

Acta Medica Scandinavica

TABLE OF CONTENTS

VOLUME 197 1975

<i>Modern medical history: The recognition of nephrogenic diabetes insipidus. A century's journey from the history of medicine</i>	1
<i>I. Turesson: Nucleolar size in benign and malignant plasma cell proliferation</i>	7
<i>A. J. Hellem, E. Segård and J. H. Solm: The adhesiveness of human blood platelets and thyroid function</i>	15
<i>E. Jakobsen, H. C. Gødal and P. Kierulf: Thalassemia minor. Twelve patients in the Norwegian families</i>	19
<i>G. Liedén, S. Höglund and L. Elm: Changes in certain iron metabolism variables after single blood donation</i>	27
<i>G. Liedén: Iron supplement to blood donors. I. Trials with intermittent iron supply</i>	31
<i>G. Liedén, S. Höglund and L. Elm: Iron supplement to blood donors. II. Effect of continuous iron supply</i>	37
<i>V. Rissanen, M. Teirka and U. Uusila: Deaths from ischaemic heart disease in Helsinki in the years 1959-1968. Vital statistics and medico-legally autopsied sudden deaths</i>	43
<i>V. Rissanen, M. Rönkä, S. Sarna and P. Sahanen: Deaths from ischaemic heart disease in persons aged 65 or younger in Helsinki in 1970. With special reference to patho-anatomic findings in hearts</i>	51
<i>J. A. Andersen, B. Flis her Hansen and K. L. Nyberg: Isolated valvular aortic stenosis. Clinicopathological findings in an autopsy material of elderly patients</i>	61
<i>K. Beck and T. Hilden: The frequency of secondary hypertension</i>	65
<i>N. Söderström, M. Teitelius-Berg and M. Ålerstam: Diagnosis of medullary carcinoma of the thyroid by fine needle aspiration biopsy</i>	71
<i>G. Järnerot: The thyroid in ulcerative colitis and Crohn's disease. I. Thyroid radioiodide uptake and urinary iodine excretion</i>	77
<i>G. Järnerot, A. K. A. Khan and S. C. Truelser: The thyroid in ulcerative colitis and Crohn's disease. II. Thyroid enlargement and hyperthyroidism in ulcerative colitis</i>	83
<i>G. Järnerot, S. C. Truelser and G. T. Warner: The thyroid in ulcerative colitis and Crohn's disease. III. The daily fractional turnover of thyroxine</i>	89
<i>G. Järnerot, S. C. Truelser and H. von Schenck: The thyroid in ulcerative colitis and Crohn's disease. IV. Thyroid hormone binding proteins</i>	95
<i>P. W. C. Kloppenborg, A. F. Casparie, T. J. Benraad and C. L. H. Majoor: Inhibition of adrenal function in man by heparin or heparinoid Ro 1-8307</i>	99
<i>P. Hedner, G. Persson and D. Ursing: Insulin release in fasting man induced by lipure but not by pure preparations of cholecystokinin</i>	

II TABLE OF CONTENTS

<i>P. Wahlberg and S.-A. Carlsson</i> Effective thyroxine ratio (ETR) and TSH as indicators in the treatment of hypothyroidism	113
<i>R. Andersson and P. Björk</i> Studies of urinary bladder dysfunction in amyloidosis with polyneuropathy	117
<i>O. Selroos and J. Edgren</i> Lupus-like syndrome associated with pulmonary reaction to nitrofurantoin. Report of three cases	125
<i>P. Telisberg</i> Complement system studies in systemic lupus erythematosus (SLE)	131
<i>J. Bichel</i> Acute leukemia in brother and sister (Follow-up)	135
<i>A. Torp</i> Myocardial biopsy in a case of cardiomyopathy and partial α -1 antitrypsin deficiency with liver enlargement	137
<i>V. Ibsen, L. Lindholm and A. Ekström</i> Erythroderma Sézary with immunological deficiency and antibodies against human albumin	141
<i>Editorial</i> Splenectomy in chronic myelocytic leukaemia	145
<i>C. O. Solberg, A. Schreiner, K. B. Hellum and E. Høy</i> Neutrophil granulocyte function in the early diagnosis of acute myelomonocytic and myeloblastic leukaemia	147
<i>I. M. Nilsson, B. Åstedt, U. Hedner and D. Berich</i> Intravascular death and circulating anticoagulant ("antithromboplastin")	153
<i>B. Lundh, E. Cavallin-Ståhl and C. Merckle</i> Heme catabolism, carbon monoxide production and red cell survival in anemia	161
<i>B. Lundh, E. Cavallin-Ståhl and C. Merckle</i> Nicotinic acid and the endogenous production of carbon monoxide	173
<i>B. Björk</i> Oxygen uptake and cardiac output during submaximal and maximal exercise in adult subjects with totally corrected tetralogy of Fallot	177
<i>B. O. Eriksson and B. Björk</i> Oxygen uptake, arterial blood gases and blood lactate concentration during submaximal and maximal exercise in adult subject with shunt-operated tetralogy of Fallot	185
<i>J. Karkkylä</i> Haemodynamic effect of atrial triggered versus fixed rate pacing at rest and during exercise in complete heart block	193
<i>D. Kasper, C. Helmers, T. Lindman and P. O. Wester</i> Initial serum potassium level in relation to early complications and prognosis in patients with acute myocardial infarction	207
<i>H. Miettinen</i> Incidence and presentation of myocardial infarction in North Karelia, Finland	211
<i>H. Ståleberg and T. Schersten</i> Substrate incorporation into hepatic lipids and proteins in vitro in patients with pre- β hyperlipoproteinemia	215
<i>K. Stærk-Johansen, T. Stærk-Johansen and J. Lærke</i> Antibody titre changes and skin reactivity in patients with liver cirrhosis undergoing portocaval shunt operation	225
<i>V. Nielsen, E. Clausen and L. Rasmussen</i> Liver impairment during chronic hemodialysis and after renal transplantation	229
<i>E. Christensen</i> Clinical histology in renal allotransplantation	235
<i>I. Callhed, B. DeLia, L. Björk, A. Hallen and L. Nordgren</i> Resection of left ventricular aneurysm—late results	41
<i>J. Fischer-Hansen, O. Pedersen-Bjergaard, P. Stage and F. Ejlert</i> Idiopathic hypertrophic subaortic stenosis	249
<i>G. Forssell, R. Nordlander, O. Åstrand and E. Östman</i> Diazepam in cardioversion	255
<i>J. Hess Thorsen, E. Christensen, A. Alarcon-Zarum and B. Morild</i> Involution of polycystic kidney during active treatment of terminal uremia	257
<i>H. E. Nielsen, H. E. Houe, B. Korsager and P. E. Sørensen</i> Renal excretion of vancomycin in kidney disease	261
<i>H. Jensen and A. W. M. Monoclonal immunoglobulinemia associated with glomerulopathy</i>	265
<i>K. Aramaki, S. Carlsson and J. I. Thorell</i> The effect of norepinephrine and theophylline on blood glucose, plasma FFA, plasma glycerol and plasma insulin in normal subjects	271
<i>B. Lund, A. Schmidt and T. Deckert</i> Portal and cubital serum insulin during oral, portal and cubital glucose tolerance test	275
<i>V. Grill and U. Rosenqvist</i> Dynamics of α -adrenergic inhibition of the adenyl cyclase—cyclic AMP system in human adipose tissue	283

<i>L. Kølster and S. Rösner</i> : Removal of exogenous triglycerides in human forearm muscle and subcutaneous tissue	299
<i>L. A. Carlsson and G. Wadellius</i> : Association between low adipose tissue content of polyunsaturated fatty acid and both glucose intolerance and hypertriglyceridemia in apparently healthy men	295
<i>E. Karlsson and L. Nohr</i> : Polymorphic acetylation of procaine amide in healthy subjects	299
<i>A. J. Joustra, M. Pasanen and M. J. Matthe</i> : Acetylator phenotype and the antihypertensive response to hydralazine	303
<i>P. O. Wester</i> : The urinary excretion of trace elements before and during treatment with hydralazine	307
<i>L. Storgaard and H. Karl</i> : Fever and haemolysis in Hodgkin's disease	311
<i>L. Brandt, I. Kövves and T. R. Möller</i> : Therapeutic effect of Leo 1031, an alkylating corticosteroid ester, in lymphoproliferative disorders. I. Chronic lymphocytic leukaemia	317
<i>T. R. Möller, L. Brandt, I. Kövves and L. G. Lindberg</i> : Therapeutic effect of Leo 1031, an alkylating corticosteroid ester, in lymphoproliferative disorders. II. Lymphocytic lymphoma	323
<i>O. S. Ørskov, M. Aas, P. Feuchtel, T. Hovig, B. Øvies, E. K. Brodwall and A. Flatmark</i> : Renin-secreting renal tumour with severe hypertension. Case report with tumour renin analysis, histopathological and ultrastructural studies	329
<i>Editorial</i> : High blood pressure and prevention of strokes	337
<i>Editorial</i> : Retrospective and prospective views on the use of viral vaccines	339
<i>H. Jørgensen, N. Norme and J. A. Sævi</i> : Scintigraphy with ¹²⁵ I-19-iodocholesterol in adrenal disease. An evaluation	345
<i>N. Daid, D. Hallberg and B. Lemle</i> : Bone mass in obese subjects	353
<i>E. Sørensen and H. Fjølsgaard</i> : Adult hypophosphataemia. Report of a case with determination of inorganic pyrophosphate in plasma and urine during high phosphate intake	357
<i>A. Berstad, H. Jørgensen, H. Frey and J. H. Vøge</i> : The acute effect of sodium cellulose phosphate on intestinal absorption and urinary excretion of calcium in man	361
<i>M. Telenius-Berg, S. Almqvist and B. Wästberg</i> : Serum calcitonin response to induced hypercalcaemia: A diagnostic aid in early occult medullary thyroid carcinoma	367
<i>B. Høe, H. Elv, G. Semb and E. Sivertsen</i> : Selective coronary arteriography	377
<i>B. Høe, H. Elv, G. Semb and E. Sivertsen</i> : Aortic coronary vein bypass in patients with angina pectoris	383
<i>A. Berner, M. Monn, P. Ørskov and A. Redfær</i> : Cardiac arrhythmias, electrolytes, and digoxin concentration in plasma and urine in patients treated with digoxin	391
<i>H. Grønneklei, M. Miller and E. Sivertsen</i> : Registration of sinus node recovery time in patients with sinus rhythm and in patients with dysrhythmias	403
<i>R. Samuelsen, L. Brønson, G. Berglund and L. Werås</i> : Minoxidil—Haemodynamic and clinical experiences with a new peripheral vasodilator	409
<i>J. Bergström and A.-M. Fridén</i> : The effect of hydrochlorothiazide and amiloride administered together on muscle electrolytes in normal subjects	415
<i>L. Pedersen, T. Aarseth and E. Hess Thyssen</i> : Turnover of plasma cholesterol in patients with cholesterol gallstones	421
<i>J. Willehelmsen</i> : Azoemia nervosa and the frequency of sex chromatin-positive cells	427
<i>J. Elmqvist, E. Johansson and C. Paul</i> : Total disappearance of fetal pulmonary emboli during streptokinase therapy of an iliofemoral thrombosis	431
<i>J. Hess Thyssen, K. Ølgaard and H. G. Jensen</i> : Ovarian cysts in women on chronic intermittent haemodialysis	433
<i>S. Ljunghall and H. Hedstrand</i> : Epidemiology of renal stones in a middle-aged male population	439
<i>U. Bengtsson, L. Hedner and C. Svalander</i> : Adult type of polycystic kidney disease in a newborn child	447
<i>S. B. Solheim, J. S. Sævi and L. Glazner</i> : The effect of spironolactone (Aldactone) and methyldopa in low and normal renin hypertension	451
<i>F. Fyhrquist, K. K. Ryyppö and M. Haavik</i> : Plasma renin activity, blood pressure and sodium excretion during treatment with clonidine	457
<i>O. Kristensen, H. Hørrestrup Andersen and J. Borup Jensen</i> : Glucose-insulin treatment of lactic acidosis in phenformin-treated diabetics	463

Minoxidil—Haemodynamic and clinical experiences with a new peripheral vasodilator (Sannerstedt, Bronson, Berglund & Werkö)	409
The smoking habits of men with intermittent claudication (Lilhell, Hedstrand & Karlsson)	473
Biopsy	
Myocardial biopsy in a case of cardiomyopathy and partial α -1 antitrypsin deficiency with liver enlargement (Torp)	137
Blood	
Thalassemia minor (Jakobsen, Godal & Kierulf)	19
Changes in certain iron metabolism variables after a single blood donation (Liedén, Höglund & Ehn)	27
Acute leukemia in a brother and sister (Bichel)	135
Splenectomy in chronic myelocytic leukaemia (Editorial)	145
Neutrophil granulocyte function in the early diagnosis of acute myelomonocytic and myeloblastic leukaemia (Solberg, Schreiner, Hellum & Hanne)	147
Intravascular death and circulating anticoagulant ("antithromboplastin") (Nilsson, Åstedt, Hedner & Berezin)	153
Heme catabolism: carbon monoxide production and red cell survival in anaemia (Lundh, Cavallin-Ståhl & Mercke)	161
Nicotinic acid and the endogenous production of carbon monoxide (Lundh, Cavallin-Ståhl & Mercke)	173
Fever and haemolysis in Hodgkin's disease (Storgaard & Karle)	311
Blood sugar	
The effect of norepinephrine and theophylline on blood glucose, plasma FFA, plasma glycerol and plasma insulin in normal subjects (Arman, Carlström & Thorell)	271
1 and cubital serum insulin during oral, portal and cubital glucose tolerance tests (Lund, & Deckert)	275
of α -adrenergic inhibition of the adenylyl cyclase—cyclic AMP system in human adipose tissue (Grill & Rosenqvist)	283
Association between a low adipose tissue content of polyunsaturated fatty acids and both glucose intolerance and hypertriglyceridaemia in apparently healthy men (Carlson & Walldius)	295
Glucose-insulin treatment of lactic acidosis in phenformin-treated diabetics (Kristensen, Harrestrup Andersen & Borup Jensen)	463
Dipping procedure for blood glucose determination with Dextrostix and the Eyetone reflectance meter (Kühl)	467
Bone	
Bone mass in obese subjects (Dahlén, Hallberg & Lanne)	333
Calcium	
The acute effect of sodium cellulose phosphate of intestinal absorption and urinary excretion of calcium in man (Berstad, Jørgensen, Frey & Vogt)	361
Serum calcitonin response to induced hypercalcaemia (Telenius-Berg, Almqvist & Wikstedt)	367
Cardioversion	
Diazepam in cardioversion (Forseell, Nordlander, Nyquist & Ormnius)	235

Catecholamines

- The effect of norepinephrine and theophylline on blood glucose, plasma PFA, plasma glycerol and plasma insulin in normal subjects (Arnsman, Carlström & Thorell) 271

CCU

- Initial serum potassium level in relation to early complications and prognosis in patients with acute myocardial infarction (Dyckner, Hehmers, Lundman & Wester) 207
- A postmyocardial infarction clinic in Göteborg, Sweden (Elmfeldt, Wilhelmsson, T. bblin, Vedin, Wilhelmsson & Bengtsson) 497

Circulation

- Deaths from ischemic heart disease in Helsinki in the years 1959-1968 (Rissanen, Teppo & Uotila) 43
- Deaths from ischemic heart disease in persons aged 65 or younger in Helsinki in 1970 (Rissanen, Romo, Sarna & Siltanen) 51
- Isolated valvular aortic stenosis (Andersen, Fischer Hansen & Lyngborg) 61
- Oxygen uptake, arterial blood gases and blood lactate concentration during submaximal and maximal exercise in adult subjects with closed-operated tetralogy of Fallot (Eriksson & Björk) 187
- Mifexidil—Haemodynamic and clinical experiences with a new peripheral vasodilator (Sauerstedt, Bronson, Berglund & Werkö) 409
- Creatine phosphokinase after submaximal physical exercise in untrained individuals (Forsell, Nordlander, Nyquist, Oksanen & Styrborn) 503

Coagulation

- Inhibition of adrenal function in man by heparin or heparinoid Ro 1-4307 (Kloppenborg, Casperis, Bernad & Major) 99
- Intrauterine death and circulating anticoagulant ("antithromboplastin") (Nilsson, Åstedt, Hedner & Berzin) 153

Collagen disease

- Lupus-like syndrome associated with pulmonary reaction to nitrofurantoin (Selroos & Edgren) 125

Complement

- Complement system studies in systemic lupus erythematosus (SLE) (Teisberg) 131

Cytology

- Nuclear size in benign and malignant plasma cell proliferation (Tunnesson) 7
- Diagnosis of medullary carcinoma of the thyroid by fine needle aspiration biopsy (Söderström, Teichner-Berg & Åkerman) 71
- Anorexia nervosa and the frequency of sex chromatin-positive cells (Wälinder) 427

Diabetes

- Glucose-ketone treatment of lactic acidosis in phenformin-treated diabetics (Krusciansen, Harstrup Andersen & Borup Jensen) 463
- Dipping procedure for blood glucose determination with De trostix and the Eytone reflectance meter (Kühn) 467

Diagnosis

- Neutrophil granulocyte function in the early diagnosis of acute myelomonocytic and myeloblastic leukaemia (Solberg, Schreiner Heflum & Hamre) 147
- Idiopathic hypertrophic subaortic stenosis (Fischer Hansen, Pedersen-Bjerggaard Stage & Efsen) 249

Diuretics

- The effect of hydrochlorothiazide and amiloride administered together on muscle electrolytes in normal subjects (Bergström & Fridén) 415
- The effect of spironolactone (Aldactone®) and methyldopa in low and normal renin hypertension (Solheim, Sandsfjord & Glazendanner) 451

Electrolytes

- The acute effect of sodium cellulose phosphate on intestinal absorption and urinary excretion of calcium in man (Berstad, Jørgensen, Frey & Vogt) 361
- Cardiac arrhythmias, electrolytes, and digoxin concentration in plasma and urine in patients treated with digoxin (Bertler, Monti, Ohlin & Redfors) 391
- The effect of hydrochlorothiazide and amiloride administered together on muscle electrolytes in normal subjects (Bergström & Fridén) 415
- Plasma renin activity, blood pressure and sodium excretion during treatment with clonidine (Fyhrquist, Kurppa & Huuskonen) 457

Endocrinology

- The recognition of nephrogenic diabetes insipidus (Editorial) 1
- The adhesiveness of human blood platelets and thyroid function (Helleim, Segård & Solem) 15
- Inhibition of adrenal function in man by heparin or heparinoid Ro 1-8307 (Kloppenborg, Casparie, Benraad & Majoor) 99
- igraphy with ¹²⁵I 19-iodocholesterol in adrenal disease (Jørgensen, Norman & Sandsfjord) 345
- calcitonin response to induced hypercalcaemia (Telenius-Berg, Almqvist & Wikstedt) 367

Enzymes

- Polymorphic acetylation of procaine amide in healthy subjects (Harrison & Molin) 299
- Acetylator phenotype and the antihypertensive response to hydralazine (Joucelo, Pasanen & Mattila) 303
- Adult hypophosphatasia (Sørensen & Flodgaard) 357
- Creatine phosphokinase after submaximal physical exercise in untrained individuals (Forssell, Nordlander, Nyquist, Orinius & Styrelius) 503

Exercise

- Creatine phosphokinase after submaximal physical exercise in untrained individuals (Forssell, Nordlander, Nyquist, Orinius & Styrelius) 503

Gastrointestinal tract

- The thyroid in ulcerative colitis and Crohn's disease. I (Järnerot) 77
- The thyroid in ulcerative colitis and Crohn's disease. II (Järnerot, Khan & Truelove) 83
- The thyroid in ulcerative colitis and Crohn's disease. III (Järnerot, Truelove & Warner) 89
- The thyroid in ulcerative colitis and Crohn's disease. IV (Järnerot, Truelove & von Schenck) 95
- Insulin release in fasting man induced by impure but not by pure preparations of cholecystokinin (Hedner, Persson & Urdahl) 109
- The acute effect of sodium cellulose phosphate on intestinal absorption and urinary excretion of calcium in man (Berstad, Jørgensen, Frey & Vogt) 361

Heart

- Deaths from ischaemic heart disease in Helsinki in the years 1959-1968 (Rissanen Tenhu & Uotila) 43
- Deaths from ischaemic heart disease in persons aged 65 or younger in Helsinki in 1970 (Rissanen Rönkä Sarma & Siltanen) 51
- Isolated valvular aortic stenosis (Andersen, Fischer Hansen & Lyngborg) 61
- Myocardial biopsy in a case of cardiomyopathy and partial α -1 antitrypsin deficiency with liver enlargement (Torp) 137
- Oxygen uptake and cardiac output during submaximal and maximal exercise in adult subjects with totally corrected tetralogy of Fallot (Björks) 177
- Oxygen uptake, arterial blood gases and blood lactate concentration during submaximal and maximal exercise in adult subjects with shunt-operated tetralogy of Fallot (Enksson & Björks) 187
- Haemodynamic effect of trial triggered versus fixed rate pacing at rest and during exercise in complete heart block (Karkk) 195
- Initial serum potassium level in relation to early complications and prognosis in patients with acute myocardial infarction (Dyckow Helmers Lundman & Wester) 207
- Incidence and presentation of myocardial infarction in North Karelia, Finland (Puska & Mustamäki) 11
- Resection of left ventricular aneurysm—late results (Cullhed Delens, Byörk, Hallén & Nordgren) 41
- Idiopathic hypertrophic subaortic stenosis (Fischer Hansen, Pedersen-Berggaard, Stage & Eftsen) 49
- Diazepam in cardioversion (Forsell, Nordlander Nyquist & Ormso) 55
- Polymorphic acetylation of procaine amide in healthy subjects (Karlsson & Molin) 299
- Selective coronary arteriography (Hoel, Eie, Semb & Sivertsen) 377
- Aortocoronary vein bypass in patients with angina pectoris (Hoel, Eie, Semb & Sivertsen) 383
- Registration of sinus node recovery time in patients with sinus rhythm and in patients with dysrhythmias (Grendahl, Müller & Sivertsen) 403
- A postmyocardial infarction clinic in Göteborg, Sweden (Elmfeldt, Wilhelmsen Tibbén Vedin Wilhelmsen & Bengtsson) 497
- Creatine phosphokinase after submaximal physical exercise in untrained individuals (Forsell Nordlander Nyquist, Ormso & Styreku) 503

Hemodialysis

- Liver impairment during chronic hemodialysis and after renal transplantation (Nielsen Clausen & Raneck) 229
- Involution of polycystic kidneys during active treatment of terminal uremia (Hess Thaysen, Christensen, Alarcon-Zarza & Movlid) 257
- Ovarian cysts in women on chronic intermittent haemodialysis (Hess Thaysen Øysaard & Jensen) 413

Hereditary

- Thalassemia minor (Jakobsen Odal & Kierulf) 19
- Acute leukemia in a brother and sister (Bache) 135
- Myocardial biopsy in a case of cardiomyopathy and partial α -1 antitrypsin deficiency with liver enlargement (Torp) 137
- Involution of polycystic kidneys during active treatment of terminal uremia (Hess Thaysen, Christensen, Alarcon-Zarza & Movlid) 257
- Adult type of polycystic kidney disease in a new-born child (Bengtsson Hedman & Svalander) 447
- Gaucher' disease and benign monoclonal gammopathy (Tresson & Rønsbo) 507

Hormones

- Insulin release in fasting man induced by insulin but not by pure preparations of (Hedöer Persson & Ursing)

X SUBJECT INDEX

Serum calcitonin response to induced hypercalcaemia (Telenius-Berg, Almqvist & Wisted)	367
Subcutaneous administration of sodium L-thyroxine to patients with hypothyroidism (Ljunggren & Persson)	471
Hypertension	
The frequency of secondary hypertension (Bech & Hildén)	65
Acetylcholinesterase and the antihypertensive response to hydralazine (Jounela, Pasanen & Mänttä)	303
The urinary excretion of trace elements before and during treatment with hydralazine (Wester)	307
Renin-secreting renal tumour with severe hypertension (Ørjaviik, Aas, Fauchald, Hovig, Øystese & Flatenmark)	329
High blood pressure and prevention of strokes (Editorial)	337
The effect of spironolactone (Aldactone®) and methyldopa in low and normal renin hypertension (Sofheim, Sandtjord & Gierendanner)	451
Plasma renin activity, blood pressure and sodium excretion during treatment with clonidine (Fyhrquist, Kurppa & Haanpää)	457
Antihypertensive effect of β -1-receptor blockade and β -2-receptor stimulation in essential hypertension (Andersson & Berghlund)	499
Immunology	
Erythrodermia <i>Sézary</i> with immunological deficiency and antibodies against human albumin (Biesma, Lindholm & Ehmst)	141
Infection	
Clinical listeriosis in renal allograft transplantation (Christensen)	235
Renal excretion of vancomycin in kidney disease (Nielsen, Hansen, Korsager & Skov)	261
Retrospective and prospective views on the use of viral vaccines (Editorial)	339
Insulin	
Insulin release in fasting man induced by impure but not by pure preparations of cholecystokinin (Hedner, Persson & Ursing)	109
The effect of norepinephrine and theophylline on blood glucose, plasma FFA, plasma glycerol and plasma insulin in normal subjects (Arman, Carlström & Thorell)	771
Portal and cubital serum insulin during oral, portal and cubital glucose tolerance tests (Læed, Schmidt & Deckert)	275
Intoxication	
The smoking habits of men with intermittent claudication (Liljebl, Hedström & Karlsson)	473
Iron metabolism	
Changes in certain iron metabolism variables after a single blood donation (Læedén, Höglund & Elm)	27
Iron supplement to blood donors. I (Læedén)	31
Iron supplement to blood donors. II (Læedén, Höglund & Elm)	27
Isotopes	
Scintigraphy with 125 I-19-iodocholesterol in adrenal disease (Jørgensen, Norman & Sundtjord)	343
Kidney	
The recognition of nephrogenic diabetes insipidus (Editorial)	1
Liver impairment during chronic hemodialysis and after renal transplantation (Nielsen, Clausen & Røsch)	229

Chronic listeriosis in renal allotransplantation (Christensen)	235
Involution of polycystic kidneys during active treatment of terminal uraemia (Hess Thaysen, Christensen, Alarcón-Zarza & Movild)	257
Renal excretion of vancomycin in kidney disease (Nielsen, Hansen, Korsager & Skov)	261
Monoclonal immunoglobulinemia associated with glomerulopathy (Jensen & Wiik)	265
The urinary excretion of trace elements before and during treatment with hydralazine (Wester)	307
Renal-secreting renal tumour with severe hypertension (Ørjaviik, Aas, Fauschold, Hovig, Øystese, Brodwall & Flatmark)	329
The acute effect of sodium cellulose phosphate on intestinal absorption and urinary excretion of calcium in man (Berstad, Jørgensen, Frey & Vogt)	361
Epidemiology of renal stones in a middle-aged male population (Ljunghall & Hedström)	409
Adult type of polycystic kidney disease in a new-born child (Bengtsson, Hedman & Svalander)	447
Plasma renin activity, blood pressure and sodium excretion during treatment with clokidone (Pyhälä, Korpja & Huuskonen)	457
Leukemia	
Acute leukemia in a brother and sister (Bichel)	135
Splenectomy in chronic myelocytic leukaemia (Editorial)	145
Neutrophil granulocyte function in the early diagnosis of acute myelomonocytic and myeloblastic leukaemia (Solberg, Schreiner, Hellum & Haure)	147
Lipids	
The effect of norepinephrine and theophylline on blood glucose, plasma FFA, plasma glycerol and plasma insulin in normal subjects (Arnesen, Carlström & Thorell)	271
Dynamics of α -adrenergic inhibition of the adenylyl cyclase—cyclic AMP system in human adipose tissue (Grill & Rosengqvist)	283
Removal of exogenous triglycerides in human forearm muscle and subcutaneous tissue (Kajfer & Rörsner)	289
Association between low adipose tissue content of polyunsaturated fatty acids and both glucose intolerance and hypertriglyceridemia in apparently healthy men (Carlson & Walldius)	295
Scintigraphy with 125 I 19-iodocholesterol in adrenal disease (Jørgensen, Norrman & Sundsfjord)	345
Turnover of plasma cholesterol in patients with cholesterol gallstones (Pedersen, Arnefred & Hess Thaysen)	421
Studies in asymptomatic primary hyperlipidaemia. II (Olsson)	477
Studies in asymptomatic primary hyperlipidaemia. III (Ekstrand & Olsson)	487
Lipoproteins	
Substrate incorporation into hepatic lipids and proteins in vitro in patients with pre- β hyperlipoproteinemia (Stakeberg & Scherström)	217
Liver	
Myocardial biopsy in case of cardiomyopathy and partial α -1 antitrypsin deficiency with liver enlargement (Torp)	137
Substrate incorporation into hepatic lipid and proteins in vitro in patients with pre- β hyperlipoproteinemia (Stakeberg & Scherström)	217
Antibody titre changes and skin reactivity in patients with liver carcinoma undergoing portocaval shunt operation (Stehr-Johansen, Stehr-Johansen & Leerhøj)	225
Liver impairment during chronic hemodialysis and after renal transplantation (Nielsen, Clausen & Rane)	229
Turnover of plasma cholesterol in patients with cholesterol gallstones (Pedersen, Arnefred & Hess Thaysen)	421

Lung

- Lupus-like syndrome associated with pulmonary reaction to nitrofurantoin (Schnoos & Edgren) 125

Lymphoma

- Fever and haemolysis in Hodgkin's disease (Storgaard & Jørgen) 311
Therapeutic effect of Leo 1031 an alkylating corticosteroid ester in lymphoproliferative disorders. I (Brundt, Könyves & Möller) 317
Therapeutic effect of Leo 1031 an alkylating corticosteroid ester in lymphoproliferative disorders. II (Möller, Brundt, Könyves & Lindberg) 323

Malformations

- Oxygen uptake and cardiac output during submaximal and maximal exercise in adult subjects with totally corrected tetralogy of Fallot (Bjarke) 177
Oxygen uptake, arterial blood gases and blood lactate concentration during submaximal and maximal exercise in adult subjects with aortopulmonary tetralogy of Fallot (Eriksson & Bjarke) 187

Medical history

- The recognition of nephrogenic diabetes insipidus (Forsman) 1

Metabolism

- Studies of urinary bladder dysfunction in amyloidosis with polyneuropathy (Andersson & Björk) 117
Amino acid catabolism, carbon monoxide production and red cell survival in anaemia (Lundh, Cavallin-Stahl & Merckel) 161
Amino acid and the endogenous production of carbon monoxide (Lundh, Cavallin-Stahl & Merckel) 173
Serum potassium level in relation to early complications and prognosis in patients with acute myocardial infarction (Dyckner, Heilmann, Lundman & Wester) 187
Substrate incorporation into hepatic lipids and proteins in vivo in patients with pre- β -hyperlipoproteinemia (Stakeberg & Schersten) 213
The effect of norepinephrine and theophylline on blood glucose, plasma FFA, plasma glycerol and plasma insulin in normal subjects (Arnesen, Carlström & Thorell) 271
Portal and cubital serum insulin during oral, portal and cubital glucose tolerance test (Lund, Schmidt & Deckert) 273
Dynamics of α -adrenergic inhibition of the adenylyl cyclase-cyclic AMP system in human adipose tissue (Grill & Rosenqvist) 283
Removal of exogenous triglycerides in human forearm muscle and subcutaneous tissue (Kajzer & Rössner) 285
Association between low adipose tissue content of polyunsaturated fatty acid and both glucose intolerance and hypertriglyceridemia in apparently healthy men (Carlson & Walldius) 293
Polymorphic acetylation of procaine amide in healthy subjects (Karlsson & Mölén) 299
Adult hypophosphatasia (Sørensen & Flodgaard) 357
Turnover of plasma cholesterol in patients with cholesterol gallstones (Pedersen, Aruffo & Henriksen) 471
Glucose-insulin treatment of lactic acidosis in phenformin-treated diabetics (Kjellgren, Hartz, Kristrup, Andersen & Børup-Jensen) 483
Studies in asymptomatic primary hyperlipidaemia. II (Olsson) 477
Studies in asymptomatic primary hyperlipidaemia. III (Ekstrand & Olsson) 487
Gaucher's disease and benign monoclonal gammopathy (Tunnesson & Rauring) 507

Methods

- Effective thyroidal ratio (ETR) and TSH as indicators in the treatment of hypothyroidism (Wahlberg & Carlsson) 113
- Oxygen uptake and cardiac output during submaximal and maximal exercise in adult subjects with totally corrected tetralogy of Fallot (Björke) 177
- Oxygen uptake, arterial blood gases and blood lactate concentration during submaximal and maximal exercise in adult subjects with shunt-operated tetralogy of Fallot (Enksson & Björke) 187
- Scintigraphy with ^{125}I 19-iodocholesterol in adrenal disease (Jørgensen, Norman & Sundsfjord) 345
- Bone mass in obese subjects (Dahlén, Hallberg & Lannek) 353
- Selective coronary arteriography (Hoch, Ele Somb & Siversten) 377
- Dipping procedure for blood glucose determination with Dextroflux and the Eytone reflectance meter (Kåhl) 467

Muscles

- Removal of exogenous triglycerides in human forearm muscle and subcutaneous tissues (Lajfer & Röster) 289
- The effect of hydrochlorothiazide and amiloride administered together on muscle electrolytes in normal subjects (Bergström & Fridén) 415

Myeloma

- Nucleolar size in benign and malignant plasma cell proliferation (Turesson) 7

Myocardial infarction

- Resection of left ventricular aneurysm—late results (Collied, Delfin, Björk, Hallén & Nordgren) 41

Nervous system

- Studies of urinary bladder dysfunction in amyloidosis with polyneuropathy (Andersson & Ejerle) 117
- Anorexia nervosa and the frequency of sex chromatin-positive cells (Wahländer) 427

Nutrition

- Anorexia nervosa and the frequency of sex chromatin-positive cells (Wahländer) 427

Obesity

- Bone mass in obese subjects (Dahlén, Hallberg & Lannek) 353

Ovary

- Ovarian cysts in women in chronic intermittent haemodialysis (Hens Thyssen, Øigård & Jensen) 433

Platelets

- The adhesiveness of human blood platelets and thyroid function (Hellein, Segård & Solem) 15

Population studies

- Deaths from ischaemic heart disease in Helsinki in the years 1959–1968 (Rissanen, Teuhu & Uotila)

Deaths from ischemic heart disease in persons aged 65 or younger in Helsinki in 1970 (Rissanen, Romo, Sarna & Siikanen)	51
The frequency of secondary hypertension (Beck & Hilden)	65
Incidence and presentation of myocardial infarction in North Karelia, Finland (Pekka & Mustaniemi)	211
High blood pressure and prevention of strokes (Editorial)	337
Epidemiology of renal stones in a middle-aged male population (Ljunghall & Hedstrand)	439
The smoking habits of men with intermittent claudication (Lithell, Hedstrand & Karlsson)	473
Pregnancy	
Intrauterine death and circulating anticoagulant ("antithromboplastin") (Nilsson, Åstedt, Hedner & Berzins)	153
Pregnosis	
A postmyocardial infarction clinic in Göteborg, Sweden (Elmfeldt, Wilhelmsson, Tibblin, Vedin, Wilhelmsson & Bengtsson)	497
Proteins	
Erythrodermia Sézary with immunological deficiency and antibodies against human albumin (Iliescu, Lindholm & Eklund)	141
Monoclonal immunoglobulinemia associated with glomerulopathy (Jensen & Wijk)	265
Gaucher's disease and benign monoclonal gammopathy (Turesson & Rauring)	307
Rehabilitation	
postmyocardial infarction clinic in Göteborg, Sweden (Elmfeldt, Wilhelmsson, Tibblin, Vedin, Wilhelmsson & Bengtsson)	497
Renal stones	
Epidemiology of renal stones in a middle-aged male population (Ljunghall & Hedstrand)	439
Skin	
Erythrodermia Sézary with immunological deficiency and antibodies against human albumin (Iliescu, Lindholm & Eklund)	141
Antibody titre changes and skin reactivity in patients with liver cirrhosis undergoing portocaval shunt operation (Stehr-Johansen, Stehr-Johansen & Leerhøj)	225
SLE	
Lupus-like syndrome associated with pulmonary reaction to nitrofurantoin (Selroos & Edgren)	125
Complement δ stem studies in systemic lupus erythematosus (SLE) (Teisberg)	131
Spleen	
Splenectomy in chronic myelocytic leukaemia (Editorial)	145
Stroke	
High blood pressure and prevention of strokes (Editorial)	337

Surgery

- Resection of left ventricular aneurysm—late results (Coffred, Dellen, Björk, Hallén & Nordgren) 241
- Aortocoronary vein bypass in patients with angina pectoris (Hool, Ele, Semb & Siverissen) 383

Thrombosis

- Total disappearance of a fatal pulmonary embolus during streptokinase therapy of an iliofemoral thrombosis (Eklund, Johansson & Paul) 431

Thyroid

- The adhesiveness of human blood platelets and thyroid function (Hållén, Segurud & Solam) 15
- Diagnosis of medullary carcinoma of the thyroid by fine needle aspiration biopsy (Söderström, Telenius-Berg & Åkerman) 71
- The thyroid in ulcerative colitis and Crohn's disease. I (Järnerot) 77
- The thyroid in ulcerative colitis and Crohn's disease. II (Järnerot, Khan & Truelove) 83
- The thyroid in ulcerative colitis and Crohn's disease. III (Järnerot, Truelove & Warner) 89
- The thyroid in ulcerative colitis and Crohn's disease. IV (Järnerot, Truelove & von Schebeck) 95
- Effective thyroxine ratio (ETR) and TSH as indicators in the treatment of hypothyroidism (Wahlberg & Carlsson) 113
- Serum calcitonin response to induced hypercalcaemia (Telenius-Berg, Åkqvist & Wiklund) 367
- Subcutaneous administration of sodium L-thyroxine to patients with hypothyroidism (Ljunggren & Persson) 471

Transplantation

- Liver impairment during chronic hemodialysis and after renal transplantation (Nielsen, Chansen & Rasek) 229
- Clinical intervals in renal allotransplantation (Christensen) 235

Treatment

- Iron supplement to blood donors. I (Liedén) 31
- Iron supplement to blood donors. II (Liedén, Höglund & Ehn) 37
- Inhibition of adrenal function in man by heparin or heparinoid Ro 1-4307 (Kloppenborg, Caspario, Bennard & Major) 99
- Lupus-like syndrome associated with pulmonary reaction to nitrofurantoin (Selroos & Edgren) 125
- Haemodynamic effect of atrial triggered versus fixed rate pacing at rest and during exercise in complete heart block (Karlöf) 195
- Antibody titre changes and skin reactivity in patient with liver cirrhosis undergoing portocaval shunt operation (Stach, Johansen, Stach, Johansen & Lærby) 225
- Diazepam in cardioversion (Forssell, Nordlander, Nyquist & Ornlöv) 255
- Renal excretion of vancomycin in kidney disease (Nielsen, II over, Korsager & Skov) 261
- Polymorphic acetylation of procaine amide in healthy subject (Karlsson & Mohn) 299
- Acetylator phenotype and the antihypertensive response to hydralazine (Jonze, Persson & Mattila) 303
- The urinary excretion of trace elements before and during treatment with hydralazine (Wester) 307
- Therapeutic effect of Leo 1031 as alkylating corticosteroid ester in lymphoproliferative disorders. I (Brandt, Könyves & Möller) 317
- Therapeutic effect of Leo 1031 as alkylating corticosteroid ester in lymphoproliferative disorders. II (Möller, Brandt, Könyves & Lindberg) 323
- Retrospective and prospective views on the use of viral vaccines (Editorial) 319
- Cardiac arrhythmias, electrolytes and digoxin concentration in plasma and urine in patients treated with digoxin (Berler, Monti, Ohlin & Redfors)

Minoxidil—Haemodynamic and clinical experiences with a new peripheral vasodilator (Sannerstedt, Brorson, Berglund & Werkö)	409
The effect of <i>h</i>)-drochlorothiazide and amiloride administered together on muscle electrolytes in normal subjects (Bergström & Fridén)	415
Total disappearance of a fatal pulmonary embolus during streptokinase therapy of an iliofemoral thrombosis (Eklund, Johansson & Paul)	431
The effect of spironolactone (Aldactone®) and methyldopa in low and normal renin hypertension (Solheim, Sundsfjord & Gjezdansky)	451
Plasma renin activity, blood pressure and sodium excretion during treatment with clonidine (Fjorquist, Kuorppa & Huusikainen)	457
Glucose-insulin treatment of lactic acidosis in phenformin-treated diabetics (Kristensen, Harsrup, Andersen & Borup Jensen)	463
Subcutaneous administration of sodium L-thyroxine to patients with hypothyroidism (Ljunggren & Persson)	471
Antihypertensive effect of β -1-receptor blockade and β -2-receptor stimulation in essential hypertension (Andersson & Berglund)	495
Tumours	
Nucleolar size in benign and malignant pleural cell proliferation (Turrisson)	7
Diagnosis of medullary carcinoma of the thyroid by fine needle aspiration biopsy (Söderström, Telenius-Berg & Åkerman)	71
Monoclonal immunoglobulinaemia associated with glomerulopathy (Jensen & Wälk)	265
Therapeutic effect of Leo 1031, an alkylating corticosteroid ester, in lymphoproliferative disorders. I (Brandt, Könyves & Möller)	317
Therapeutic effect of Leo 1031, an alkylating corticosteroid ester, in lymphoproliferative disorders. II (Möller, Brandt, Könyves & Lindberg)	323
Renin-secreting renal tumour with severe hypertension (Orjani, Aas, Panchald, Hovig, Dystase, Brodwall & Flatmark)	329
Ovarian cysts in women on chronic intermittent haemodialysis (Hess Thaysen, Olgaard & Jensen)	433
Tract	
Studies of urinary bladder dysfunction in amyloidosis with polyneuropathy (Andersson & Bjerke)	117
Virus	
Retrospective and prospective views on the use of viral vaccines (Editorial)	139

LIST OF AUTHORS

- Åkerman, M. 71
 Aas, M. 329
 Åstedt, B. 153
 Alarcos-Zurita, A. 257
 Almqvist, S. 367
 Andersen, J. A. 61
 Andersson, O. 495
 Andersson, R. 117
 Arnfred, T. 421
 Arason, K. 271
 Axnick, N. Suppl. 576
- Beale, A. J. Suppl. 576
 Beck, K. 63
 Bengtsson, C. 497
 Bengtsson, U. 447
 Besrand, T. J. 99
 Berezin, D. 153
 Berglund, G. 409 495
 Bergström, J. 415
 Berlin, R. 145
 Berstad, A. 361
 Bertler, Å. 391
 Bichel, J. 135
 Björke, B. 177 187
 Björke, P. 117
 Björk, L. 241
 Borup Jensen, J. 463
 Brandt, L. 317 323
 Broadwall, E. K. 329
 Bronson, L. 409
- Carlson, L. A. 295
 Carlsson, S. A. 113
 Carlström, S. 271
 Casparie, A. F. 99
 Cavallin-Stihl, E. 161 173
 Christensen, E. 235 257
 Claassen, E. 229
 Cockburn, W. Chas. Suppl. 576
 Croac, C. Suppl. 578
 Culthed, L. 241
- Dahlén, O. Suppl. 570
 Dalén, N. 353
 Deckert, T. 275
 Della, W. 241
 van Dijk, H. Suppl. 572
 Detzel, J. Suppl. 578
 Dyckner, T. 207
- Edgren, J. 125
 Efsen, F. 249
 Elm, L. 27 37
- Ehrnst, A. 141
 Elm, H. 377 383
 Ekstrand, L.-G. 487
 Ekstrand, J. 431
 Elmfeldt, D. 497
 Eriksson, B. O. 187
- Færevold, P. 329
 Fischer Hansen, B. 61
 Fischer Hansen, J. 249
 Flatmark, A. 329 Suppl. 571
 Flodgaard, H. 357
 Forsaell, G. 255 303
 Forsman, H. 1
 Frits, E. D. Suppl. 576
 Fry, H. 361
 Fridén, A.-M. 415
 Fyhrquist, F. 457
- Garby, L. Suppl. 578
 Gard, S. Suppl. 576
 Glenzendorfer, L. 451
 Godal, H. C. 19
 Grendahl, H. 403
 Gresser, I. Suppl. 576
 Gröb, V. 283
 Gurrum, K. A. Suppl. 571
- Hallberg, D. 353
 Hallén, A. 241
 Hamre, E. 147
 Hansen, H. E. 261
 Harrestrup Andersen, H. 463
 Hedman, L. 447
 Hedner, P. 109
 Hedner, U. 153
 Hedström, H. 439 473
 Hellén, A. J. 15
 Hellén, K. B. 147
 Hellén, C. 207
 Hess Thøysen, E. 421
 Hess Thøysen, J. 257 433
 Hildén, T. 65
 Håkansson, M. R. Suppl. 576
 Höglund, S. 27 37
 Hock, H. 377 383
 Hovig, T. 329 Suppl. 571
 Husby, O. Suppl. 571
 Huuskonen, M. 457
- Ilicu, V. 141
- Järnerot, G. 77 83 89 95
 Jakobsen, E. 19
- Jansen, H. Suppl. 571
 Jensen, H. O. 265 433
 Jørgensen, H. 345 361
 Johansson, E. 431
 Jönck, A. J. 303
- Kaljer, L. 289
 Karlén, H. 311
 Karlén, L. 195
 Karlsson, E. 299
 Karlsson, R. 473
 Karzon, D. T. Suppl. 576
 Khan, A. A. A. 83
 Klemm, P. 19
 Kilbourne, E. D. Suppl. 576
 Kloppenborg, P. W. C. 99
 Kåmyres, I. 317 323
 Kohner, E. M. Suppl. 578
 Korsager, B. 261
 Kristensen, O. 463
 Karypa, J. 457
 Köhl, C. 467
- Laitinen, O. Suppl. 577
 Lamke, B. 353
 Larsen, H. W. Suppl. 578
 Leerby, J. 225
 Liden, G. 27 31 37
 Lindberg, L. G. 323
 Lindholm, L. 141
 Litell, H. 473
 Ljunggren, J. G. 471
 Ljungvall, S. 439
 Lund, B. 275
 Lundh, B. 161 173
 Lundman, T. 207
 Lundström, R. Suppl. 576
 Lyngberg, K. 61
- Majoor, C. L. H. 99
 Mattila, M. J. 303
 Melton, J. H. Suppl. 578
 Mercke, C. 161 173
 Miller, M. 403
 Möller, T. R. 317 323
 Mohr, L. 299
 Mont, M. 391
 Morris, J. N. Suppl. 576
 Movik, B. 257
 Mostafaei, H. 211
 Myhre, E. Suppl. 571
- Nielsen, H. E. 261
 Nielsen, V. 229

MODERN MEDICAL HISTORY

THE RECOGNITION OF NEPHROGENIC DIABETES INSIPIDUS

A Very Small Page from the History of Medicine

The history of medicine is replete with the names of Hippocrates, Galen and Avicenna, to say nothing of Lister and Semmelweis. In its way this article also deals with medical history though it is concerned only with a very small piece of observation, bearing little significance for mankind if one takes the broad view. It recounts in anecdotal form the recognition of two new genotypical clinical entities both characterized by the symptom of diabetes insipidus. The historical perspective is very short—little more than 30 years. In scientific matters anecdotal material is often regarded with some contempt. My own view is a different one. It seems to me that we can learn a good deal from anecdotes and strange coincidences may hold the seeds of scientific observation—seeds which deserve to be allowed to take root.

However, I would not have ventured to think of publishing the matter even as a small contribution to the history of medicine had not the Editor of this Journal requested me to do so. He was present at a medical meeting and heard an informal talk of which this article is a part. The fact that this fragment appears in print is entirely the fault of Professor Jan Waldenström. It began in this way:

In April 1940 the German armies occupied Sweden's neighbour country Norway and a large number of Swedish hospital doctors were called up for military service. Although not then fully qualified I was requested by the Chief of the Medical Department of the University Hospital, Uppsala, Professor Gustaf Bergmark, to serve as relief house physician. One of my first duties in this post concerned a man with diabetes insipidus. A doctor in the provincial medical service—just as young as I and also acting as relief—telephoned to get some information from a hospital case paper. He was attending a male patient E. K. who obviously had diabetes since

there was polyuria and sugar was present in the urine. On the other hand the urinary specific gravity was low and the output volume exceedingly high. Did the patient have diabetes mellitus or diabetes insipidus? The answer was that he had both—a lifelong sufferer from diabetes insipidus had acquired a mild diabetes mellitus of maturity type.

Study of the patient's case record revealed in the main the information about him and his siblings set out in Fig. 1 in which E. K. is no 9 in generation III. He was one of 13 siblings—eight men and five women. Diabetes insipidus had affected certainly four and possibly a fifth of the eight brothers but not one of the five sisters. In the evening I looked up the available genetic textbooks. They stated that diabetes insipidus in its genetic form was inherited as an autosomal dominant character and the main reference quoted was the gigantic German genealogical tree which had been published by Adolph Wei, (7) his son Alfred Wei (8) and the latter's son-in-law Camerer (1).

However, a dominant autosomal transmission accorded badly with my set of siblings. If four (or perhaps five) of eight brothers get the disease but not even one of five sisters, this raises strong suspicion rather of a sex-linked transmission. On the basis of this single set of siblings alone it was justifiable to conclude that here a dominant transmission carrying equal risk for the two sexes was highly improbable.

I searched old case record and visited a number of relatives of the 13 brothers and sisters. An old brother of E. K.'s mother born in 1854 (II.2) came to light and his diabetes insipidus was recorded in hospital case papers. Two sons of one of E. K.'s sisters were also traced, both had been treated in

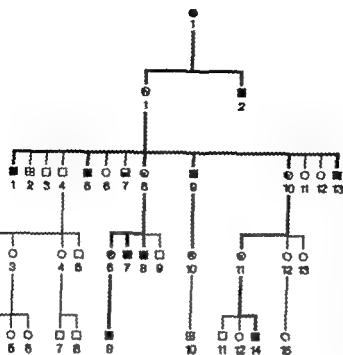


Fig 1 Pedigree representing nephrogenic diabetes insipidus, inherited as a recessive X-chromosome-linked trait. □=healthy male, ○=healthy female ■=affected male, ◻=possibly affected male, ○=conductor, ⊙=unknown individual in earlier generation presumably gene bearer, ♂♂=ended in early infancy

hospital for diabetes insipidus. The sex-linked transmission became quite obvious.

The secretary of the department where I was working noticed that I was searching for cases of familial diabetes insipidus. She told me that her other had friends in Tierp, a small town north of psala, and that in the family of these friends there was a series of persons with diabetes insipidus. Was there then another stock and if so was it connected in any way with E. K.'s family? There followed a visit to an old lady in Tierp. She telephoned at once to her middle-aged son who himself suffered from diabetes insipidus, though he had never been treated in hospital. However, there was his maternal grandfather and sure enough I found that he figured in the case records of the University Hospital with this diagnosis. The old lady had a family bible in which the names of a series of relatives were recorded. Mother and son sat with me, pointed to various names and told me who had suffered from "the thirst" and who had not. It was exclusively men that they reported as affected and in this family the pattern of X-chromosome linkage was clear from the start. The family tree stretched away to Dalecarlia in central Sweden—there seemed to be no end to it. As the genealogical tangle unravelled the family became overwhelmingly large. Fig. 2 shows a single one of eight

branches of a genealogical tree which four generations further back originated in a common woman ancestor born in 1712. However it also became clear from the genealogical study that no connection could be shown between the K. siblings and the new dynasty from Tierp and Dalecarlia.

The line of clinical study picked its way forward in the following way. In the thirties, amidopyrine had been used in the therapy of diabetes insipidus. The rationale of this treatment was based on an observation by an Austrian, D. Scherf, that amidopyrine blocks the effect of mercurial diuretics in the treatment of oedema. Two patients with acquired diabetes insipidus who were under treatment in our Medical Department clearly received some benefit from amidopyrine therapy. E. K. and one of his brothers had been given amidopyrine but neither of them showed the least response to this substance. However, in the forties we had available various preparations of the antidiuretic hormone. When E. K. was 11 years old, a trial was made of vasopressin, pituitrin and tonephin but even with large doses not the least effect was discernible on urinary volume, chloride output or specific gravity. A son of one of E. K.'s sisters willingly cooperated in a trial of the same preparations. He was a youth of 17 years, mentally slightly backward but physically healthy. In his case



Fig 2 Pedigree representing pituitary diabetes insipidus, inherited as recessive, X-chromosome-linked trait. Symbols as in Fig 1

there was no reason to consider impaired renal function due to age or any other cause. Like his uncle E. K., he showed no vestige of effect from the antidiuretic hormone. As biological proof of the effectiveness of the preparation we divided the contents of the ampoule and gave half to a healthy volunteer: he showed a powerful antidiuretic effect.

At this time a woman with previously known diabetes insipidus had been admitted to the Maternity Department for the birth of her third child. Study of her family tree revealed dominant autosomal transmission that is the type described in the textbooks. This form of the disease is undoubtedly much more common and I now know four such families in Sweden. Some kind members of this last family made the journey to undergo trial of the antidiuretic hormone. All three who were investigated responded with a pronounced and typical antidiuretic effect.

The hypothesis now appeared to be temptingly simple: there was one form of diabetes insipidus inherited by autosomal dominant transmission, which responded to the antidiuretic hormone. There was also another form, inherited as a sex chromosome-linked recessive which was refractory to the hormone.

Study of the literature brought out the long-known fact that hereditary diabetes insipidus affects men more often than women. This had led to some rather far-fetched speculations about the pathophysiology of the disease. It was thought that testosterone played some part in the physical manifestation of

the gene. A German Lickint had written a paper entitled "Ist der Diabetes Insipidus eine genito-hypophysäre Erkrankung?" Lickint gave the answer "yes" to his own question, his principal argument being the pronounced excess of males among those with the disease. His paper was highly speculative but it nevertheless did supply a stimulus. If the disease exists in different forms and if one of these forms is inherited by X-linked recessive transmission, then this could be the whole explanation of the excess of males found in the various case series. For example Hanhart (4) compiled a group of family trees with a total of 169 cases of the disease. Of these 108 were males representing a significant deviation from the expected proportion 50:50.

It was necessary to compile a series made up entirely of undoubted cases of autosomal transmission. This could be done by including only those from family trees which showed transmission of the disease from father to son, an impossibility with X-linked inheritance. I collected from the literature 13 pedigrees of this type: this produced 90 males and 63 females. In any such compilation there is a selection factor involved which overweighs the males, i.e. the requirement of inheritance from father to son. Those two members in each family who are responsible for this selection effect must be subtracted and when this correction is made the sex distribution becomes 64 males against 63 females. There was no longer any point in speculating about the effect of testos-

sterone on pathophysiology. At all events the preponderance of males is fully explained by the existence of X-linked genes in certain families.

During this time new events had occurred on the clinical side. It had to be confirmed that the X-linked recessive gene always implies a refractory state towards the antidiuretic hormone. A number of men from many parts of the country kindly agreed to be admitted to our Medical Department or to be investigated at their local hospitals for a trial of the hormone. The majority belonged to the huge family tree which had its origin in Dalecarlia. The transmission was unquestionably X-chromosome-linked recessive. Nine men of this lineage were tested with pituitrin. All nine showed a prompt response, thus refuting the hypothesis. In other words the neatly streamlined explanation postulating two genetic patterns and two clinical forms was incorrect.

However, from the original smaller family of E. K. and his 12 siblings four members could be tested with the hormone and all proved to be refractory. It gradually became evident that this family represented a separate form of the malady which also had its own special character among other things a much more severe type of disease. Further study made it possible to define two different X-chromosome-linked genetic disorders with diabetes insipidus, which had not previously been distinguished. Like the commoner autosomal and dominant inherited form of the disease,

one of these X-linked forms responds to the antidiuretic hormone. In fact no clinical difference can be demonstrated between these two distinct genetic entities with their different types of transmission. The other X-linked form of the disease is what later came to be termed renal or nephrogenic diabetes insipidus. It is refractory to the hormone and so must have a completely different pathophysiology.

Among the special risks of the nephrogenic form of the disease must be included the risk of mental retardation. This association became clear to me only after my thesis on diabetes insipidus (3) was already in press. However quite early on one mother had told the following story. At a very early age her first son was remarkably thirsty. The family doctor advised restriction of the child's naughty drinking. The boy had constant fever and his bowel function was extremely sluggish. His development ceased, he became emaciated and he died at the age of 18 months. Seven years later this woman had her second son and everything began in the same way.

She was again advised to restrict fluids but this time she disobeyed the instructions and gave the boy as much water as he wanted. He is still alive, healthy and able to work but he is backward and had to attend an ESN school. This account of the two brothers perhaps contains a good deal of suggestion but as will appear later the suggestion is not without justification.

During Christmas 1943 I received a visit from an old friend the paediatrician Edgar Mannheimer who later died during his service as professor and head of the Ethiopian Swedish Paediatric Clinic in Addis Ababa. We were talking about diabetes insipidus and Mannheimer said that in his children's hospital in Stockholm he had recently seen an extraordinary boy aged two years. The child had protracted fever without any signs of infection for months he had had a swinging temperature often very high. His growth and development had stagnated and he had chronic constipation, with faeces resembling mortar. Gradually it was noted that the child was avid for water and could consume astonishing quantities. The diagnosis of diabetes insipidus had ultimately been made but only after long delay. When he finally got a liberal supply of water his alarming condition subsided and he began to flourish. Mannheimer happened to mention the boy's surname which is a very uncommon one. It made me start, for this name was very familiar to me from E. K.'s family. There was no difficulty in establishing that the boy was a son of the daughter of one of E. K.'s sisters (V 9 in Fig. 1).

The head of the Children's Clinic, Crown Princess Lovisa's Hospital in Stockholm was Professor Adolf Lichtenstein. I was able to show him a case paper from the University Hospital in Uppsala written in 1905 by the recently qualified house physician Adolf Lichtenstein and referring to a patient who was a brother of the maternal grandmother of the boy then under treatment in the Crown Princess Lovisa's Hospital (III 5). The case paper had been written with great care and it recorded a history taken from relatives which described the patient's swinging fever, emaciation and severe constipation at an early age. The descendant at that time under Lichtenstein's care recovered well and was discharged a few months after he began to receive liberal amounts of water. However he remained mentally backward and received his schooling in an institution for the mentally retarded.

Yet another coincidence taught me to recognize the paediatric picture of nephrogenic diabetes insipidus. In the winter of 1944-45 while reading the proofs of my thesis I was called up for the civil defence service at a place in the far north of Sweden. We had to care for those of the civil population who had been removed from the evacuated areas during the final hostilities between Finland and Russia, mainly mothers and children. I was responsible for an emergency hospital and for the paediatric work. I had the help of the chief of a nearby children's hospital. One evening this colleague told me that he was worried about a most unusual case in his hospital: a 1 year-old boy who seemed likely to die. He had high swinging fever without any other signs of infection, wasting and severe constipation. That boy has diabetes insipidus, I declared and I got the very reasonable reply that my proof reading had gone to the brain and that there really were some other kinds of disease too! We hurried to the children's hospital. The clinical history was strikingly similar to that of the boy at Professor Lichtenstein's hospital in Stockholm two years earlier. I asked if I might telephone the mother of the child and my colleague agreed though he thought it rather odd. I asked the mother whether she had any relatives with the unusual name of the previous boy. The answer was "yes" she had cousins of that name down in the Uppsala-Stockholm region and she herself was born just outside Uppsala. Lichtenstein's patient and this boy (V 14 Fig. 1) were second cousins, their mothers were first cousins and their maternal grandmothers were both sisters of E. K.

We returned to the boy and gave him liberal drinks of water. Next day he was afebrile and remained so. *H* began to gain weight and about a month later he was discharged from the hospital in good health. However he was found to be mentally retarded and later attended school in an institution for the mentally deficient.

Nephrogenic diabetes insipidus is nowadays included in the textbooks as a cause of mental retardation. It is also clear that if the diagnosis is not recognized in time there is risk of early death. In couple of later publications I put forward the view that cerebral damage results from early dehydration, either directly or through some toxic effect. The litre of milk which forms the routine feed of me for young infants cannot replace these infants' water loss. If the mother is known heterozygote all her

male children should be plied with water until they reach an age at which the diagnosis can be confirmed or excluded.

This sketch of the way in which the observations on the two different forms of X-chromosome-linked hereditary diabetes insipidus meandered their way forward through a series of coincidences is not recalled mainly from memory. I still have detailed notes and photo-copies of the original case records.

In 1945 that is in the same year as my thesis appeared Waring, Kajli and Tappan (6) published quite independently of me their observation of nephrogenic diabetes insipidus in six boys of whom three were half-brothers. They described the clinical picture in early infancy and pointed out that the antidiuretic hormone I without effect.

This then was one of those coincidences of which one is wont to use the expression 'the time was ripe'. It is clear that the observation was made independently in the USA and in Sweden. I published it in a preliminary form in 1942 (7) and therefore had no knowledge of the paper by Waring and his colleagues of 1945. My paper of 1942 was published in a Swedish language journal although with a short summary in English. It is not reasonable to suppose that the American workers could have been acquainted with it.

At the time of their discovery many new disease entities appear to be extremely rare. The fact that all my patients could soon be shown to belong to the same family also gave this impression. Later it became clear—and this is how things usually are—that nephrogenic diabetes insipidus is not so extraordinarily rare. I have been in contact with two other families in Sweden who are affected by this disease and it is known in many other parts of the world.

Hans Forsman, University of Gothenburg, Psychiatric Research Centre St. Jürgen Hospital, S-422 03 Hisingers Backa, Sweden

REFERENCES

1. Camerer J W. Eine Ergänzung des Weischen Diabetes insipidus-Stammbaumes. Arch. Rassenbiol. 25: 382, 1935.
2. Forsman, H. Om ärftlighetslagen III diabetes insipidus (On the mode of hereditary transmission diabetes insipidus). Nord Med. 16: 3211, 1942.
3. — On hereditary diabetes insipidus with special regard to sex-linked form. Acta med. scand. Suppl. 199, 1945.
4. Hanhart, E. Die Erbsytopathologie des Diabetes insipidus.

- 1a. *Handbuch der Erbologie des Menschen* Vol. IV 2 (ed. G. Jusi). Springer Berlin 1940
- 5 Lickint F. Ist der Diabetes insipidus eine genito-hypophysäre Erkrankung? *Dtsch. med. Wochr.* 60: 1672 1934
6. Waring, A. J., Kajdi, L. & Tappan V. A congenital defect of water metabolism. *Amer. J. Dis. Child.* 69: 323, 1945
- 7 Weil Adolph. Über die hereditäre Form des Diabetes insipidus. *Arch. Anat.* 95 70, 1884
8. Weil Alfred. Über die hereditäre Form des Diabetes insipidus. *Dtsch. Arch. kln. Med.* 93 180 1908.

NUCLEOLAR SIZE IN BENIGN AND MALIGNANT PLASMA CELL PROLIFERATION

Ingemar Turesson

*From the Department of Internal Medicine, University of Lund
Malmö General Hospital, Malmö, Sweden*

Abstract Nucleolar and nuclear size of bone marrow plasma cells has been studied in 26 cases of myeloma, 19 of benign essential monoclonal gammopathy (BEMG) and 9 without an M-component. Bone marrow smears were Feulgen-stained to visualize nucleoli and nuclei. Nucleolar area, nuclear area and the ratio nucleolar area/nuclear area were calculated. The myeloma group differed from the other two groups in having plasma cells with larger nucleoli, larger nuclei and an increased ratio nucleolar area/nuclear area. No difference was found between the BEMG group and the cases without an M-component. In some cases—in which the absence of osteolytic lesions at X-ray examination, low plasma cell percentage in bone marrow smears or low M-component concentration initially made the diagnosis of myeloma uncertain—the observation of enlarged nucleoli could help to establish the diagnosis. Three cases were observed in which the onset of myeloma was preceded by a long period of essentially unchanged M-component concentration. In these cases sudden increase of M-component concentration was accompanied by an increase of mean nucleolar size of bone marrow plasma cells.

Multiple myeloma is a disease characterized by a monoclonal proliferation of immunoglobulin-producing cells in the bone marrow. Their product can be demonstrated as an M-component in serum and/or Bence Jones protein in urine. M-components also occur however in persons without clinical, cytological, or radiological signs of multiple myeloma, as was first pointed out by Waldenström (16). The term benign essential monoclonal gammopathy (BEMG) has later been applied to this condition (18). While multiple myeloma is a malignant tumor which, if untreated, grows until the death of its host, BEMG can be regarded as a benign tumor which has not quite escaped the homeostatic control of proliferation. At a certain size the growth of the tumor is slowed down so that a steady state is

achieved. BEMG occurs in 1% of the population over 25 years of age and in about 3% of people over 70 years. Its incidence is about 40 times that of myeloma (2). When an M-component is discovered it is thus essential to decide whether the underlying condition is a myeloma, a macroglobulinemia Waldenström or a BEMG. This is all the more important as we have now a treatment for myeloma and macroglobulinemia which is effective but far from being without danger.

The differential diagnosis of multiple myeloma and BEMG can be difficult. In early stages of multiple myeloma osteolytic bone lesions can often not be demonstrated by X-ray. In general myeloma differs from BEMG in having more plasma cells in bone marrow smears, higher concentration of M-component in serum, and larger quantities of monoclonal light chains in urine. There is however a wide overlap between the two conditions in these respects (9). In many cases the only way to decide whether myeloma or BEMG is present is continued observation of the patient. An unchanged concentration of the M-component for several years is a strong indication of BEMG (9, 17). The aim of this investigation was to find out whether morphological differences between the plasma cells in BEMG and myeloma can facilitate the establishment of the differential diagnosis of these two conditions.

MATERIAL

The material consists of 54 patients, 26 with myeloma, 19 with BEMG and 9 without an M-component (Table I). Three cases of myeloma with a long static phase before the outbreak of signs of typical myeloma are reported separately. All cases have been examined in the Department of

Table 1 Description of the material

No M-component	9
a Polyclonal Ig increase	4
b Normal Ig concentration	5
BEGM	19
a M-component type IgG	13
b M-component type IgA	5
c M-component type IgM	1
Myeloma	26
a M-component type IgG	17
b M-component type IgA	6
c Bence Jones protein	3
BEGM developing into myeloma type IgG	3

Medicine Malmö General Hospital during 1965-71. Four of the cases without an M-component had a polyclonal immunoglobulin increase, while five had normal immunoglobulin concentrations. Eight BEGM cases were selected because of plasma cell percentages above 5% in bone marrow smears. All other cases were unselected.

The diagnostic criteria for multiple myeloma were as follows. 1) M-component in serum and/or Bence Jones protein in urine. 2) Osteolytic bone lesion demonstrated by X-ray (not only osteoporosis) without any other apparent explanation than a plasma cell proliferation (more than 3% plasma cells in bone marrow smears) or macroscopic lytic bone lesions demonstrated at autopsy corresponding to a proliferation of plasma cells.

Cases fulfilling the following criteria were classified as BEGM. 1) M-component in serum. 2) No osteolytic bone lesions at X-ray examination and/or no macroscopic lytic bone lesions at autopsy. 3) Unchanged M-component concentration or an increase at repeated examination of not more than 0.5 g/100 ml during an observation period of at least three years.

Most cases were under observation for far more than three years. When the study was interrupted, 13 patients were dead. All but one of these were autopsied. Two cases who showed no signs of myeloma at autopsy were accepted as BEGM even though the observation period was slightly shorter than three years. Six patients were still alive at the end of the study. The average observation period for all BEGM cases was seven years. In two cases the M-component disappeared during the observation period. In all myeloma cases except five bone marrow examination was done before cytostatic therapy was started. In the BEGM cases bone marrow examination was done when the M-component was discovered in some and later during the observation period in others.

METHODS

Bone marrow was aspirated by sternal puncture and smears were made by conventional slide technique. Slides were stained with May-Grunwald-Giemsa and 1000 cells were counted to calculate the plasma cell percentage. To demonstrate nucleoli, slides were Feulgen-stained after fixation in absolute methanol for 10 min (6). Contrast staining of the cytoplasm with light green was used to



Fig. 1 Bone marrow plasma cells in a case of BEGM (a) and a case of myeloma (b). Feulgen light green. $\times 1600$.

facilitate the identification of plasma cells. With this technique it was possible to demonstrate in the majority of the plasma cells a single round or oval, eccentrically placed nucleolus, separated from surrounding nuclear DNA by a membrane-like structure of condensed chromatin (Fig. 1). Intraneuclear inclusions occurred in some myeloma cells but were as a rule easily distinguished from nucleoli. With a Zeiss ocular micrometer two diameters of nucleolus and of nucleus were measured, and the areas were calculated using the formula for the area of an ellipse. If two or more nucleoli occurred in the same cell the areas were lumped together. In each slide 25-30 cells were measured and the means of nucleolar area, nuclear area and relative nucleolar area (nucleolar area/nuclear area $\times 100$) were calculated. By measuring 25 cells S.E.M. of less than 5% was obtained. In varying number of plasma cells (10-20%) the demarcation of the nucleolus from the nucleus was not quite sharp and these cells were omitted. There was no correlation between the number of such cells and mean nucleolar or nuclear size.

Protein studies were made in the Department of Clinical Chemistry. M-component concentrations were estimated in most cases by paper electrophoresis and protein determination after separate elution of the M-component (7).

Table II. Nucleolar area, nuclear area, relative nucleolar area and plasma cell percentage in 9 patients without an M-component

Pat. no.	Diagnosis	Plasma cell percentage	Nucleolar area (μ^2)		Nuclear area (μ^2)		Relative nucleolar area	
			Mean	S.D.	Mean	S.D.	Mean	S.D.
1	Rheum. arthritis	2.0	5.3	1.1	51.3	9.3	10.4	2
2	Monocytic leukemia	2.7	5.8	2.0	60.2	11	9.6	3.0
3	Collagenosis	1.1	5.2	1.1	59.4	12.5	8.8	1.3
4	Rheum. arthritis	2.1	5.8	1.9	53.8	11.5	11.0	3.1
5	Fly's syndrome	1.9	6.3	1.9	59.0	10.7	10.7	7
6	Coccydionia acuta	1.6	5.7	1.4	63.0	11.3	9.1	2.1
7	Myocardial inf.	2.7	4.2	1.1	50.3	10.4	8.4	1.8
8	Renal cancer	2.9	5.9	1.2	61.5	13.3	9.9	1.9
9	Cancer of uterine cervix	1.0	5.1	1.3	62.9	12.3	8.2	1.6
Mean		2.1	5.5		57.9		9.6	
S.D.		0.7	0.6		4.9		1.0	

9). In some cases electroimmunoelectrophoresis according to Laurell (10) was used. Classification of M-components was made using immunoelectrophoresis with monospecific antisera.

RESULTS

The values for mean nucleolar area, nuclear area, relative nucleolar area, and plasma cell percentage in 9 cases without an M-component are given in Table

II. If the measured values from all cases are pooled (370 cells) typical normal variation curves are obtained for nucleolar as well as nuclear area. The number of measured cells in each case is too small to allow a definite statement of normal variation. However, no tendency to a bimodal variation was observed. Tables III and IV show the corresponding values for the BEMG and the myeloma cases.

Table III. Nucleolar area, nuclear area, relative nucleolar area and plasma cell percentage in 19 BEMG patients

G = light chain type not determined

Pat. no.	M-component		Plasma cell percentage	Nucleolar area (μ^2)		Nuclear area (μ^2)		Relative nucleolar area	
	Type	Conc. (g/100 gel)		Mean	S.D.	Mean	S.D.	Mean	S.D.
1	GK	1.6	3.9	7.2	3.1	62.4	7.0	12.2	4.2
	GK	1.0	3.7	5.0	1.5	48.5	9.7	10.4	1.9
3	GK	0.8	6.1	4.5	1.0	58.6	12.0	7.9	1.8
4	GK	2.8	11.0	5.2	1.5	65.9	14.0	8.0	1.7
5	GK	1.2	9.5	7.2	2.6	56.8	10.6	12.5	3.2
6	GK	1.7	6.6	4.9	1.1	80.0	9.4	6.2	1.2
7	GK	1.1	0.7	3.7	0.9	52.0	9.1	7.3	1.3
8	GL	1.7	3.0	6.8	1.2	57.7	21.5	11.3	1.4
9	GL	1.3	3.7	6.5	1.4	59.5	10.2	11.0	2.2
10	GL	1.1	3.3	7.2	2.4	61.1	10.4	11.5	9
11	G	2.0	5.4	4.5	1.0	48.2	8.8	9.4	2.1
12	G	1.2	4.6	4.2	0.9	50.6	10.9	8.4	1.7
13	G	1.2	3.3	3.6	1.0	37.5	5.9	9.7	2.3
14	AK	0.8	2.6	10.2	6	93.1	18.2	10.8	1.6
15	AK	0.6	3.4	7.5	1.6	58.2	9.4	13.0	2.2
16	AK	0.8	2.3	4.8	1.7	46.0	12.6	10.2	2.5
17	AK	1.6	1.8	5.3	1.8	66.6	14.8	8.3	2.6
18	AK	0.6	1.5	4.8	1.9	55.5	13.4	8.7	1.9
19	Mh	0.9	5.6	5.3	1	51.7	10.1	10.5	2.3
Mean		1.3	4.3	5.7		58.5		9.9	
S.D.		0.6	2.6	1.6		1.4		1.9	

- In. Handbuch der Erbbiologie des Menschen Vol. IV 2 (ed. G. Jast) Springer Berlin 1940
- 5 Lickint, F. Ist der Diabetes insipidus eine geräthypophysäre Erkrankung? Dtsch. med. Wochschr. 60: 1672 1934
- 6 Waring, A. J. Kajdi, L. & Tappan V. A congenital defect of water metabolism. Amer. J. Dis. Child. 69: 323 1945
- 7 Weil, Adolph. Über die hereditäre Form des Diabetes insipidus. Arch. Anat. 95 70 1884
8. Weil, Alfred. Über die hereditäre Form des Diabetes insipidus. Dtsch. Arch. klin. Med. 93 180 1908.

NUCLEOLAR SIZE IN BENIGN AND MALIGNANT PLASMA CELL PROLIFERATION

Ingemar Turtason

*From the Department of Internal Medicine, University of Lund
Malmö General Hospital, Malmö, Sweden*

Abstract Nucleolar and nuclear size of bone marrow plasma cells has been studied in 26 cases of myeloma, 19 of benign essential monoclonal gammopathy (BEMG) and 9 without an M-component. Bone marrow smears were Feulgen-stained to visualize nucleoli and nuclei. Nucleolar area, nuclear area and the ratio nucleolar area/nuclear area were calculated. The myeloma group differed from the other two groups in having plasma cells with larger nucleoli, larger nuclei and an increased ratio nucleolar area/nuclear area. No difference was found between the BEMG group and the cases without an M-component. In some cases—in which the absence of osteolytic lesions at X-ray examination, low plasma cell percentage in bone marrow smears or a low M-component concentration initially made the diagnosis of myeloma uncertain—the observation of enlarged nucleoli could help to establish the diagnosis. Three cases were observed in which the onset of myeloma was preceded by long period of essentially unchanged M-component concentration. In these cases a sudden increase of M-component concentration was accompanied by an increase of mean nucleolar size of bone marrow plasma cells.

Multiple myeloma is a disease characterized by a monoclonal proliferation of immunoglobulin-producing cells in the bone marrow. Their product can be demonstrated as an M-component in serum and/or Bence Jones protein in urine. M-components also occur however in persons without clinical cytological or radiological signs of multiple myeloma, as was first pointed out by Waldenström (16). The term benign essential monoclonal gammopathy (BEMG) has later been applied to this condition (18). While multiple myeloma is a malignant tumor which, if untreated, grows until the death of its host, BEMG can be regarded as a benign tumor which has not quite escaped the homeostatic control of proliferation. At a certain size the growth of the tumor is slowed down so that a steady state is

achieved. BEMG occurs in 1% of the population over 25 years of age and in about 3% of people over 70 years. Its incidence is about 40 times that of myeloma (2). When an M-component is discovered it is thus essential to decide whether the underlying condition is a myeloma, a macroglobulinemia Waldenström or a BEMG. This is all the more important as we have now a treatment for myeloma and macroglobulinemia which is effective but far from being without danger.

The differential diagnosis of multiple myeloma and BEMG can be difficult. In early stages of multiple myeloma osteolytic bone lesions can often not be demonstrated by X-ray. In general myeloma differs from BEMG in having more plasma cells in bone marrow smears, higher concentration of M-component in serum and larger quantities of monoclonal light chains in urine. There is however a wide overlap between the two conditions in these respects (9). In many cases the only way to decide whether myeloma or BEMG is present is continued observation of the patient. An unchanged concentration of the M-component for several years is a strong indication of BEMG (9, 17). The aim of this investigation was to find out whether morphological differences between the plasma cells in BEMG and myeloma can facilitate the establishment of the differential diagnosis of these two conditions.

MATERIAL

The material consists of 54 patients, 26 with myeloma, 19 with BEMG and 9 without an M-component (Table I). Three cases of myeloma with long static phase before the outbreak of signs of typical myeloma are reported separately. All cases have been examined in the Department of

Table 1 Description of the material

No M-component	9
a Polyclonal Ig increase	4
b Normal Ig concentration	5
BEGM	19
a M-component type IgG	13
b M-component type IgA	5
c M-component type IgM	1
Myeloma	26
a M-component type IgG	17
b M-component type IgA	6
c Bence Jones protein	3
BEGM developing into myeloma type IgG	3

Medicine Malmö General Hospital, during 1965-71. Four of the cases without an M-component had a polyclonal immunoglobulin increase while five had normal immunoglobulin concentrations. Eight BEGM cases were selected because of plasma cell percentages above 5% in bone marrow smears. All other cases were unselected.

The diagnostic criteria for multiple myeloma were as follows: 1) M-component in serum and/or Bence Jones protein in urine. 2) Osteolytic bone lesions demonstrated by X-ray (not only osteoporosis) without any other apparent explanation than plasma cell proliferation (more than 3% plasma cells in bone marrow smears) or macroscopic lytic bone lesions demonstrated at autopsy corresponding to proliferation of plasma cells.

Cases fulfilling the following criteria were classified as BEGM: 1) M-component in serum. 2) No osteolytic bone lesions at X-ray examination and/or no macroscopic lytic bone lesions at autopsy. 3) Unchanged M-component concentration at repeated examination of not less than 0.5 mg/100 ml during an observation period of at least three years.

Most cases were under observation for far more than three years. When the study was interrupted, 13 patients were dead. All but one of these were autopsied. Two cases, who showed no signs of myeloma at autopsy were accepted as BEGM even though the observation period was slightly shorter than three years. Six patients were still alive at the end of the study. The average observation period for all BEGM cases was seven years. In two cases the M-component disappeared during the observation period. In all myeloma cases except five bone marrow examination was done before cytostatic therapy was started. In the BEGM cases bone marrow examination was done when the M-component was discovered in some and later during the observation period in others.

METHODS

Bone marrow was aspirated by sternal puncture and smears were made by conventional slide technique. Slides were stained with May-Grunwald-Giemsa and 1000 cells were counted to calculate the plasma cell percentage. To demonstrate nucleoli, slides were Feulgen-stained after fixation in absolute methanol for 10 min (6). Contrast staining of the cytoplasm with light green was used to



Fig. 1 Bone marrow plasma cells in a case of BEGM (a) and case of myeloma (b). Feulgen light green. $\times 1600$.

facilitate the identification of plasma cells. With this technique it was possible to demonstrate in the majority of the plasma cells a single, round or oval, eccentrically placed nucleolus, separated from surrounding nuclear DNA by a membrane-like structure of condensed chromatin (Fig. 1). Intracellular inclusions occurred in some myeloma cells but were, as a rule, easily distinguished from nucleoli. With Zeiss ocular micrometer two diameters of nucleolus and of nucleus were measured and the areas were calculated using the formula for the area of an ellipse. If two or more nucleoli occurred in the same cell the areas were lumped together. In each slide 25-30 cells were measured and the means of nucleolar area, nuclear area and relative nucleolar area (nucleolar area/nuclear area $\times 100$) were calculated. By measuring 25 cells S.E.M. of less than 5% was obtained. In varying number of plasma cells (10-20%) the demarcation of the nucleolus from the nucleus was not quite sharp and these cells were omitted. There was no correlation between the number of such cells and mean nucleolar or nuclear area.

Protein studies were made in the Department of Clinical Chemistry. M-component concentrations were estimated in most cases by paper electrophoresis and protein determination after separation of the M-component (7).

Table II Nucleolar area, nuclear area, relative nucleolar area and plasma cell percentage in 9 patients without an M-component

Pat. no.	Diagnosis	Plasma cell percentage	Nucleolar area (μ^2)		Nuclear area (μ^2)		Relative nucleolar area	
			Mean	S.D.	Mean	S.D.	Mean	S.D.
1	Rheum. arthritis	2.0	5.3	1.1	51.3	9.3	10.4	2
2	Monocytic leukemia	1.7	5.8	2.0	60.2	11.2	9.6	3.0
3	Collagenosis	1.1	5.1	1.1	39.4	12.5	8.8	1.3
4	Rheum. arthritis	1.1	5.8	1.9	53.8	11.5	11.0	3.1
5	Felty syndrome	1.9	6.3	1.9	39.0	10.7	10.7	2.7
6	Condyloma acan.	6	5.7	1.4	63.0	11.3	9.1	1
7	Myocardial inf.	2.7	4.2	1.1	40.3	10.4	8.4	1.8
8	Renal cancer	2.9	5.9	1	61.5	13.3	9.9	1.9
9	Cancer of uterine cervix	1.0	5.1	1.3	62.9	12.3	8.2	1.6
Mean		2.1	5.5		57.9		9.6	
S.D.		0.7	0.6		4.9		1.0	

9). In some cases electroimmunoresay according to Laurell (10) was used. Classification of M-components was made using immunoelectrophoresis with monospecific antisera.

RESULTS

The values for mean nucleolar area, nuclear area, relative nucleolar area and plasma cell percentage in 9 cases without an M-component are given in Table

II. If the measured values from all cases are pooled (370 cells) typical normal variation curves are obtained for nucleolar as well as nuclear area. The number of measured cells in each case is too small to allow a definite statement of normal variation. However, no tendency to a bimodal variation was observed. Tables III and IV show the corresponding values for the BEMG and the myeloma cases.

Table III. Nucleolar area, nuclear area, relative nucleolar area and plasma cell percentage in 19 BEMG patients

G = light chain type not determined

Pat. no.	M-component		Plasma cell percentage	Nucleolar area (μ^2)		Nuclear area (μ^2)		Relative nucleolar area	
	Type	Conc. (g/100 ml)		Mean	S.D.	Mean	S.D.	Mean	S.D.
1	GK	1.6	3.9	7.2	3.1	62.4	7.0	1.2	4.2
2	GK	2.0	3.7	5.0	1.5	48.5	9.7	10.4	1.9
3	GK	0.8	6.1	4.5	1.0	58.6	12.0	7.9	1.8
4	GK	2.8	11.0	5.2	1.5	65.9	14.0	8.0	1.7
5	GK	1.2	9.5	7.2	2.6	46.8	10.6	12.5	3.2
6	GK	1.7	6.6	4.9	1.1	80.0	9.4	6.1	1.2
7	GK	1.2	0.7	3.7	0.9	52.0	9.1	7.3	1.3
8	GL	1.7	3.0	6.8	2.2	57.7	21.5	11.3	1.4
9	GL	1.3	3.7	6.5	1.4	59.5	10.2	11.0	2.2
10	GL	1.1	3.3	7.2	4	62.1	10.4	11.5	9
11	G	2.0	5.4	4.5	1.0	48.2	8.8	9.4	2.1
12	G	1.2	4.6	4	0.9	50.6	10.9	8.4	1.7
13	G	1.2	3.3	3.6	1.0	37.5	5.9	9.7	2.3
14	AK	0.8	2.6	10.2	2.6	93.1	18	10.8	1.6
15	AK	0.6	3.4	7.5	1.6	58.2	9.4	13.0	1.2
16	AK	0.8	3	4.8	1.7	46.0	12.6	10.2	2.5
17	AK	1.6	1.8	5.3	1.8	66.6	14.8	8.3	2.6
18	AK	0.6	1.5	4.8	1.9	55.5	13.4	8.7	1.9
19	MK	0.9	5.6	5.3	1.2	51.7	10.1	10.5	2.3
Mean		1.3	4.3	5.7		58.5		9.9	
S.D.		0.6	2.6	1.6		12.4		1.9	

Table IV Nucleolar area, nuclear area, relative nucleolar area and plasma cell percentage in 26 myeloma patients

BJ = Bence Jones proteinuria, III = light chain type not determined, n.d. = not determined

Pat. no.	M-component			Plasma cell percentage	Nucleolar area (μ^2)		Nuclear area (μ^2)		Relative nucleolar area	
	Conc. (g/100 ml)	BJ			Mean	S.D.	Mean	S.D.	Mean	S.D.
1	GK	4.3	Trace	40	14.0	2.3	84.5	9.0	16.7	2.9
2	GK	1.6	Pos.	22	12.3	3.1	79.3	14.2	15.8	3.1
3	GK	4.0	Neg.	13	14.6	3.4	84.8	12.0	17.3	3.8
4	GK	1	Trace	3	12.8	4.3	89.6	18.3	14.2	4.3
5	GK	0.4	Neg.	2	7.1	3.4	73.1	15.3	9.6	3.9
6	GK	1.5	n.d.	10	17.8	7.1	94.3	28.9	19.0	5.8
7	GK	4.0	Pos.	27	5.9	1.4	69.9	10.9	8.3	1.8
8	GK	1.9	Trace	8	10.3	3.4	81.2	15.3	13.5	3.4
9	GK	2.3	Neg.	20	15.1	3.1	96.1	14.3	15.9	3.6
10	GK	7.0	n.d.	11	17.3	4.4	90.9	22.8	19.3	4.6
11	GK	3.2	Pos.	9	18.1	7.9	104.4	50.8	16.4	3.6
12	GL	4.8	Pos.	22	9.1	1.9	70.5	17.9	13.1	2.2
13	GL	2.6	Trace	3	9.7	2.7	84.6	17.7	11.7	2.6
14	GL	6.4	Pos.	25	10.2	3.2	69.6	18.3	14.7	3.3
15	GL	6.3	Pos.	5	11.8	3.8	55.6	7.4	21.3	4.2
16	II	3.4	Pos.	8	11.2	4.5	83.5	28.5	13.6	4.3
17	III	4.7	n.d.	8	8.0	1.4	66.5	13.0	12.3	2.0
18	AK	4.9	Neg.	28	14.1	3.1	98.9	15.6	17.6	2.9
19	AK	3.2	Pos.	25	12.0	4.3	75.1	12.5	15.7	1.9
20	AK	4.0	Neg.	12	11.4	4.3	85.1	25.3	13.8	1.6
21	AK	2.4	Neg.	10	14.1	4.0	98.1	19.1	14.6	3.7
22	AK	2.1	Trace	9	12.2	3.7	83.8	17.4	14.6	2.0
23	AL	1.2	Pos.	7	21.1	6.9	100.7	19.1	20.6	5.1
24	K	-	Pos.	12	13.4	3.8	78.5	14.1	17.0	3.4
25	L	-	Pos.	45	12.5	3.7	60.6	19.4	1.1	3.2
26	L	-	Pos.	30	11.4	3.9	72.9	17.0	15.8	4.9
Mean	3.4			15.9	12.6		81.2		15.5	
S.D.	1.7			11.5	3.5		14.0		3.2	

Figs 2, 3 and 4 demonstrate nucleolar area, nuclear area and relative nucleolar area for the three groups of patients. Each point represents the mean of 25-50 measured cells in one slide. In three cases of myeloma with a long benign prephase the cells were measured at the stage when the M-component was discovered (the condition was then judged as BEMG) and in the first bone marrow aspirate made after a sudden increase in the M-component concentration was registered (the condition was then classified as myeloma).

It is evident that the myeloma group differs from the other groups in having plasma cells with larger nucleoli and larger nuclei. The ratio nucleolar area/nuclear area is also increased. The differences are statistically significant ($p < 0.001$) for these three variables. The best separation of the myeloma group is obtained using nucleolar area. On the other hand there is no difference between the group

without an M-component and the BEMG group.

The plasma cell percentages in bone marrow smears of the three diagnostic groups are demonstrated in Fig. 5. Again there is good separation between groups but overlapping is more prominent. In the three cases with a long benign stage, cell measurements were made several times during the course and the results are given in Fig. 6.

DISCUSSION

It is a well known fact that myeloma cells can differ from normal plasma cells in several respects: one or more large nucleoli, large nuclei of varying form, increased nucleo-cytoplasmic ratio, occurrence of binucleated cells, loose chromatin structure in the nuclei etc. (9, 17). Myeloma cases with predominantly normal "mature" plasma cells are, however, not uncommon. Fadern (7). In a series of

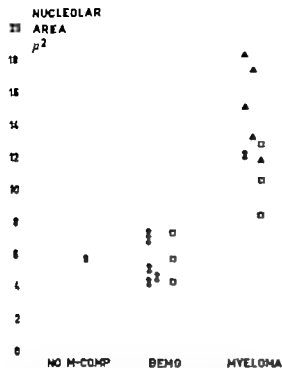


Fig. 2. Mean nucleolar area in bone marrow plasma cells in myeloma, BEMO and various diseases without an M-component. \circ = no cytostatic therapy Δ = after induction of cytostatic therapy \square = BEMO developing into myeloma, no cytostatic therapy

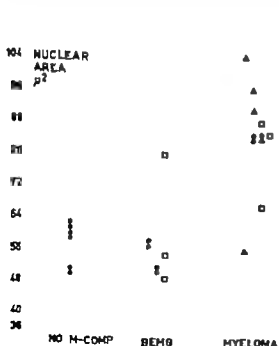


Fig. 3. Mean nuclear area in myeloma, BEMO and various diseases without an M-component. Symbols as in Fig. 2.

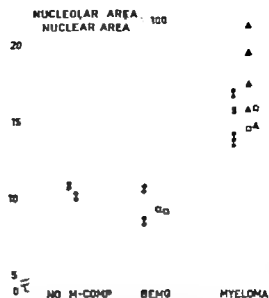


Fig. 4. Nucleolar area as percentage of nuclear area in bone marrow plasma cells in myeloma, BEMO and various diseases without an M-component. Symbols as in Fig. 2.

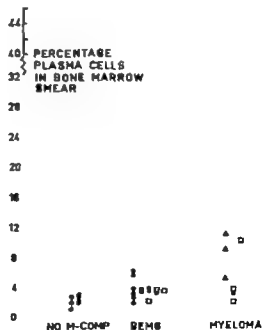


Fig. 5. Plasma cell percentage in bone marrow smears in myeloma, BEMO and various diseases without an M-component. Symbols as in Fig. 2.

Table IV Nucleolar area, nuclear area, relative nucleolar area and plasma cell percentage in 26 myeloma patients

BJ = Bence Jones proteinuria, G = light chain type not determined n.d. = not determined

Pat. no.	M-component			Plasma cell percentage	Nucleolar area (μ^2)		Nuclear area (μ^2)		Relative nucleolar area	
	Conc. (g/100 ml)	BJ			Mean	S.D.	Mean	S.D.	Mean	S.D.
1	GK	4.5	Trace	40	14.0	2.3	84.5	9.0	16.7	2.9
2	GK	1.6	Pos.	22	12.3	3.1	79.3	14.2	15.8	3.1
3	GK	4.8	Neg.	13	14.6	3.4	84.8	12.0	17.3	3.8
4	GK	2.1	Trace	3	12.8	4.3	89.6	18.3	14.2	4.3
5	HK	0.4	Neg.	2	7.1	3.4	73.1	15.3	9.6	3.9
6	GK	1.5	n.d.	10	17.8	7.1	94.3	28.9	19.0	5.8
7	GK	4.0	Pos.	27	5.9	1.4	69.9	16.9	8.5	1.8
8	GK	1.9	Trace	8	10.3	3.4	81.2	15.3	13.5	3.4
9	GK	2.3	Neg.	20	15.1	3.1	96.1	14.5	15.9	3.6
10	GK	7.0	n.d.	11	17.3	4.4	90.9	22.8	19.5	4.6
11	GK	3.2	Pos.	9	18.1	7.9	104.4	50.8	16.4	3.6
12	OL	4.8	Pos.	22	9.1	1.9	70.5	17.9	13.1	2.2
13	OL	4.6	Trace	3	9.7	2.7	84.6	17.7	11.7	2.6
14	OL	6.4	Pos.	10	10.2	3.2	69.6	18.3	14.7	3.3
15	OL	6.3	Pos.	5	11.8	3.8	55.6	7.4	21.3	4.0
16	U	3.4	Pos.	8	11.2	4.5	83.5	28.5	13.6	4.3
17	O	4.7	n.d.	8	8.0	1.4	66.5	13.6	12.3	2.8
18	AK	4.0	Neg.	28	14.1	3.1	80.9	15.6	17.6	2.9
19	AK	3.2	Pos.	25	12.0	4.3	75.1	12.5	15.7	1.9
20	AK	4.0	Neg.	12	11.4	4.3	85.1	25.3	13.8	1.6
21	AK	2.4	Neg.	10	14.1	4.0	96.1	19.1	14.6	3.7
22	AK	2.1	Trace	9	12.2	3.7	83.8	17.4	14.6	2.8
23	AL	1.2	Pos.	7	21.1	6.9	100.7	19.1	20.6	5.1
24	K	-	Pos.	12	13.4	3.8	78.5	14.1	17.0	3.4
25	L	-	Pos.	45	12.3	3.7	66.6	19.4	21.1	3.2
26	L	-	Pos.	30	11.4	3.9	72.9	17.0	15.8	4.9
Mean	3.4			15.9	12.6		81.2		15.5	
S.D.	1.7			11.5	3.5		14.0		3.2	

Figs 2, 3 and 4 demonstrate nucleolar area, nuclear area and relative nucleolar area for the three groups of patients. Each point represents the mean of 25-50 measured cells in one slide. In three cases of myeloma with a long benign prephase the cells were measured at the stage when the M-component was discovered (the condition was then judged as BEMG) and in the first bone marrow aspirate made after a sudden increase in the M-component concentration was registered (the condition was then classified as myeloma).

It is evident that the myeloma group differs from the other groups in having plasma cells with larger nucleoli and larger nuclei. The ratio nucleolar area/nuclear area is also increased. The differences are statistically significant ($p < 0.001$) for these three variables. The best separation of the myeloma group is obtained using nucleolar area. On the other hand there is no difference between the group

without an M-component and the BEMG group.

The plasma cell percentages in bone marrow smears of the three diagnostic groups are demonstrated in Fig. 5. Again there is good separation between groups but overlapping is more prominent. In the three cases with a long benign stage cell measurements were made several times during the course and the results are given in Fig. 6.

DISCUSSION

It is a well known fact that myeloma cells can differ from normal plasma cells in several respects, one or more large nucleoli, large nuclei of varying form, increased nucleio-cytoplasmic ratio, occurrence of binucleated cells, loose chromatin structure in the nuclei, etc. (9, 17). Myeloma cases with predominantly normal mature plasma cells are however not uncommon. Faden (7) in a series of

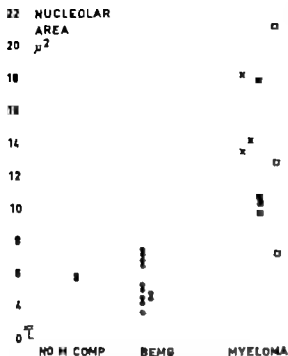


Fig 7 Mean nucleolar area in bone marrow plasma cells in various diseases without an M-component, BEMG and myeloma without osteolytic lesions at first X-ray examination. ● BEMG or no M-component, ■ myeloma with M-component conc. >2.0 g/100 ml and plasma cells in bone marrow smear $>7\%$, □ myeloma with M-comp conc. <2.0 g/100 ml or plasma cells in bone marrow smear $<7\%$, × myeloma with M-comp conc. <2.0 g/100 ml and plasma cells in bone marrow smear $<7\%$.

52 consecutive cases of myeloma found no less than 17 such cases. Likewise Hillén (9) found plasma cells of normal appearance in several cases of myeloma and atypical plasma cells in a few cases of BEMG. Ahern (1) on the other hand who used Feulgen staining for demonstration of nucleoli found an increased nucleolar/nuclear ratio in 10 cases of myeloma as compared in 10 cases of reactive plasmacytosis.

The present investigation clearly demonstrates that bone marrow plasma cells in myeloma with few exceptions differ from other plasma cells by having larger nucleoli, larger nuclei and an increased nucleolar/nuclear ratio. It can be objected that it is not possible with ordinary light microscopy to decide which plasma cells belong to the M component-producing clone and which do not. However Hjörns et al (8) have shown by immunofluorescence that in myeloma as well as in

BEMG the majority of bone marrow plasma cells contain the same class of heavy and light chain as those forming the M-component. The present author has been able to confirm these results and found that they are valid also in cases with a low M-component concentration and that good agreement is obtained between bone marrow aspirates from different sites in one and the same patient (unpublished observations).

Enlarged nucleoli and increased nucleolus/nucleus ratio are common in malignant cells. They are however not restricted to malignant cells but can be seen in growing or protein-secreting non-neoplastic cells (5, 6). It has not been possible to demonstrate any consistent differences in nucleolar ultrastructure between malignant and normal cells (4). As in other blood cell series the maturation from plasma blast to plasma cell is accompanied by a gradual decrease of nucleolar size. Smetana et al (14) have shown, by specifically staining RNA containing structures that the nucleoli of normal mature plasma cells are small and have a ring-formed structure in contrast to the large and compact nucleoli of immature plasma cells. Electron-microscopic studies have confirmed that ribonucleoprotein components are distributed in the periphery of such ring-formed nucleoli but uniformly in compact nucleoli. The presence of large and compact nucleoli in myeloma cells with well developed rough endoplasmic reticulum has been interpreted as a sign of maturation asynchrony of these cell components (13).

The structural observations can be viewed in conjunction with autoradiographic studies demonstrating a very low degree of RNA synthesis in normal mature plasma cells but a varying degree of persistent RNA synthesis in a high proportion of myeloma cells (11, 12). Furthermore, studies of aminonucleoside inhibition of ribosomal RNA synthesis in HeLa cells have led to the conclusion that the shape and size of the nucleolus are determined by the balance between the rate of the production of the precursor of ribosomal RNA by the nucleolar regions of the chromosome and the demand for ribosomal RNA by the cytoplasm (15). Quaglini et al (11), in their autoradiographic studies of myeloma cells and normal plasma cells found no evidence of a higher degree of protein synthesis in myeloma cells. In addition they observed that only a small proportion (2–10%) of the myeloma cell population did synthesize DNA.

In most cases estimation of nucleolar and nuclear area does not give further information of vital importance for the diagnosis than the combination of serum and urine protein electrophoreses, radiological examination of the skeleton, and examination of Pappenheim-stained bone marrow smears. There are however some cases where the observation of enlarged nucleoli can make an earlier diagnosis of myeloma possible. Fig. 7 shows a number of cases in which the absence of osteolytic lesions at X-ray examination, a low plasma cell percentage in bone marrow smear or a low M-component concentration initially made the diagnosis of myeloma uncertain. All but one of these cases had enlarged nucleoli when the M-component was discovered and all subsequently developed all criteria for the diagnosis of multiple myeloma.

The present material is too small to allow any definite conclusion as to whether nucleolar area has any prognostic value in myeloma. It should be pointed out, however, that the 5 cases of myeloma with nucleolar areas less than $10 \mu^2$ all had remarkably long survival times. Two of them are still alive with observation periods of 82 and 89 months and the three who died had survival times of 71, 73 and 75 months respectively. The mean survival time for the whole material was 42 months.

Three cases were observed in which the onset of myeloma was preceded by a long period of essentially unchanged M-component concentration during 5–10 years. These cases should be regarded as cases of BEMG being transformed into myeloma. The M-component remained the same type after transformation but increased rapidly in concentration. It was not possible to sort out these cases from the other BEMG cases by measuring nucleolar area during the "benign" stage. When the M-component concentration increased there was a parallel increase in mean nucleolar area. This might be explained by a new clone with a higher degree of malignancy arising out of the benign clone. In one case bone marrow smear was available shortly before a sudden increase of M-component concentration was observed. Measurement of a large number of cells showed an asymmetric curve with an increase in the number of cells with large nucleoli. It was however not possible to demonstrate a definitive bimodal variation.

REFERENCES

1. Abernethy W. A. The differentiation of myelomatosis from other causes of bone marrow plasmacytosis. *J. clin. Path.* 11: 326, 1958.
2. Axelsson, U., Bachmann, R. & Hällén, J. Frequency of pathological proteins (M-components) in 5995 sera from an adult population. *Acta med. scand.* 179: 235, 1966.
3. Bachmann, R. & Laurell C.-B. Electrophoretic and immunologic classification of M-components in serum. *Scand. J. clin. Lab. Invest. Suppl.* 69: 11, 1963.
4. Bernhard W. Some problems of fine structure in tumor cells. *Progr. exp. Tumor Res.* 3: 1, 1963.
5. Busch, H., Byrvoet, P. & Smetana K. The nucleolus of the cancer cell. A review. *Cancer Res.* 31: 313, 1963.
6. Busch, H. & Smetana K. The nucleolus. Academic Press, New York and London, 1970.
7. Faden, R. Differentiation of plasmacytic responses from myelomatous diseases on the basis of bone marrow findings. *Cancer* 5: 128, 1952.
8. Hjalmar, W., Schult, H. R. & Hühling, Hesseink, E. An immunofluorescence study on intracellular immunoglobulins in human bone marrow cells. *Ann. N.Y. Acad. Sci.* 177: 290, 1971.
9. Hällén, J. Discrete gammaglobulin (M-) components in serum. Clinical study of 150 subjects without myelomatosis. *Acta med. scand., Suppl.* 462, 1966.
10. Laurell C.-B. Electrophoretic and electroimmunological analysis of proteins. *Scand. J. clin. Lab. Invest. Suppl.* 124, 1972.
11. Quaglini, E., Torelli, U., Sanli, S. & Mauri, C. Cytochemical and autoradiographic investigations on normal and myelomatous plasma cells. *Acta haematol.* 38: 79, 1967.
12. Schmid, J., Kieley, J., Tanta, W. & Owen, Ch. In vitro DNA and RNA synthesis in human bone marrow cells. A study of 12 normal subjects and 12 patients with lymphoplasmaic disorders. *Blood* 27: 310, 1966.
13. Smetana, K., Gyorky, F., Gyorky, Ph. & Busch, H. Ultrastructural studies on human myeloma plasmacytes. *Cancer Res.* 33: 2300, 1973.
14. Smetana, K., Lane, M. & Busch, H. Studies of nucleoli of leukemic granulocytes and of plasmacytes in multiple myeloma. *Exp. molec. Path.* 5: 236, 1966.
15. Studzinski, G. & Gierthy, J. Cytologic appearance of the nucleolus of normal and neoplastic cells in relation to the synthesis of RNA. *Acta cytol.* 16: 245, 1972.
16. Waldenström, J. Abnormal proteins in myeloma. *Advanc. Intern. Med.* 5: 398, 1952.
17. — Diagnosis and treatment of multiple myeloma. Grune and Stratton, New York, 1970.
18. — Benign monoclonal gammopathies. In: Multiple myeloma and related disorders (ed. H. A. Azar and M. Potter). Harper and Row, New York, 1973.

THE ADHESIVENESS OF HUMAN BLOOD PLATELETS AND THYROID FUNCTION

Arvid J. Hellem, Erik Segård and Jan H. Soløm

*From the Department of Internal Medicine Akerhus Central Hospital
University of Oslo Oslo Norway*

Abstract. Hypothyroidism is associated with severe coronary atherosclerosis. In spite of this the reported incidence of angina pectoris and myocardial infarction in untreated hypothyroidism is small. Since many authors consider the formation of thrombus in coronary arteries to be the final event of the process which leads to myocardial infarction, changes in the platelet function may explain the paradoxical rarity of myocardial infarction in untreated hypothyroidism. To evaluate this hypothesis, platelet adhesiveness has been estimated before and after treatment in 9 hypothyroid and 16 thyrotoxic patients. In thyrotoxicosis the platelet adhesiveness was significantly increased, but decreased to normal after treatment. In hypothyroidism platelet adhesiveness was abnormally low but increased to normal values after thyroid hormone replacement. This may be an important factor in precipitating myocardial infarction in patients with hypothyroidism and coronary artery atherosclerosis.

Patients with thyrotoxicosis and hypothyroidism are in an exceptional position with regard to coronary heart disease. Extensive clinical and necropsy studies have shown that severe coronary atherosclerosis occurs twice as frequently in patients with myxoedema as in controls matched for age, sex, BP and associated extra thyroidal disorders (18). Despite hypercholesterolaemia and increased atherosclerosis the incidence of myocardial infarction in untreated myxoedema is not increased. When thyroxin is given to such patients the incidence of angina pectoris and the risk of precipitating myocardial infarction increases (19).

In hyperthyroidism the plasma cholesterol level is low but the incidence of atherosclerosis is unknown. However an incidence of angina pectoris from 10 to 12% has been reported (1). Consequently it should be expected that a coincidental occurrence of myocardial infarction and thyrotoxicosis would

not be a rarity. In an unselected series of 384 patients suffering from thyrotoxicosis 7 developed myocardial infarction during an active stage of thyrotoxicosis, and in a further 8 there appeared signs of acute coronary disease suggestive of mild myocardial infarction (4). Thus the general impression of the infrequency of myocardial infarction in active thyrotoxicosis seems not to be valid.

The profound influence of thyroid dysfunction on metabolic processes is well recognized but its relation to platelet adhesiveness has been sparingly investigated (16-17). The first stage in thrombus formation is the adhesion of platelets to the injured vessel wall and to each other leading to the formation of the white thrombus. As thromboembolic disorders have been reported to be associated with increased platelet adhesiveness (7-14) it seemed important to determine whether alterations in thyroid function were associated with changes in platelet adhesiveness.

PATIENTS AND METHODS

Nine hypothyroid and 16 thyrotoxic patients have been examined with regard to the adhesiveness of blood platelets by the glass bead filter method of Hellem (9) before, during and after treatment. The main principle of the method is to expose native blood to standardized glass bead columns. By an electrically driven mechanical device the blood from a graduated syringe is forced through the glass bead column at constant rate. The difference of the platelet count before and after passage through the column gives the number of adhesive platelets. This is expressed as percentage of the initial count. The normal value by this method is 75% with S.D. of 13. In addition the primary bleeding time has been estimated in 6 hypothyroid patients and in all the thyrotoxic patients by modification of Ivy method (2). Normal value <12 min.

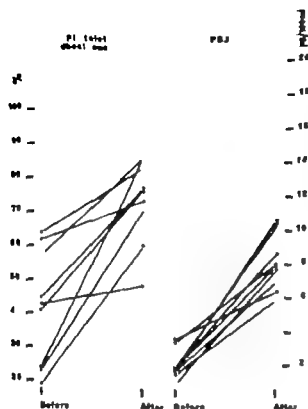


Fig 1 Percent adhesive platelets and PBJ before and after treatment in 9 hypothyroid patients.

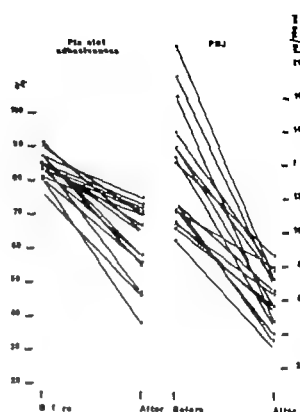


Fig 2 Percent adhesive platelets and PBJ before and after treatment in 16 thyrotoxic patients.

RESULTS

Hypothyroidism

Fig 1 shows platelet adhesiveness and PBJ before and after treatment in the 9 hypothyroid patients. The mean platelet adhesiveness was 43% before and 73% after treatment. This difference is highly significant ($p < 0.001$). All the patients showed low initial values which increased during treatment to euthyroid state. The mean platelet number was 206 000 before and 226 000 after treatment. This difference is not significant. The mean bleeding time in 6 of these patients was 10 min before and 6 min after treatment ($p < 0.05$).

Thyrotoxicosis

According to Fig 2 all the thyrotoxic patients showed an initial high adhesiveness which decreased to normal values during treatment to euthyroid state. This is the opposite change from what was seen in the 9 hypothyroid patients. The mean platelet adhesiveness was 84% before and 62% after treatment. This difference is also highly significant ($p < 0.001$).

The mean platelet number was 231 000 before and 250 000 after treatment. This difference is not significant. The mean bleeding time remained unchanged after treatment (6½ min).

DISCUSSION

It appears that the hypothyroid patient is in some way spared from the effects of the accompanying arterial disease until thyroxin is administered. The decreased platelet adhesiveness in hypothyroidism may explain the reduced incidence of myocardial infarction in spite of the extensive atherosclerotic changes in their coronary arteries. However other mechanisms may also be involved in this protection. Several investigations have shown that the fibrinolytic activity of the blood is increased in hypothyroidism (1, 10, 11). In these reports it is suggested that the depression of the fibrinolytic activity after administration of thyroxin may facilitate the formation of an occluding thrombus in the coronary arteries. In addition, decreased activity of the blood intrinsic coagulation system has been

demonstrated in the hypothyroid state (5-6). These abnormalities are reversed by administration of thyroxine. Also a slight bleeding tendency has been reported in myxoedema in spite of normal platelet count (5) indicating a qualitative platelet dysfunction.

In contrast, decreased fibrinolytic activity and increased activity of the intrinsic coagulation system have been demonstrated in hyperthyroidism (6-10). A slight reduction of the number of circulating platelets and a shortening of the platelet life span has also been reported indicating an increased peripheral trapping of the platelets in thyrotoxicosis (12).

From a theoretical point of view these abnormalities enhance the risk of formation of platelet thrombus. It has been claimed that the hyperthyroid patients show a low incidence of myocardial infarction (8-13). This has been attributed to the antiatherogenic effect of the thyroid hormone demonstrated in cholesterol-fed rabbits (15). Furthermore the thyroid hormones reduce the serum cholesterol level and increase the coronary blood flow which will reduce the local thrombus formation. Despite these reducing factors Burnstein *et al.* (4) found in their series the incidence of myocardial infarction not to be less than that of a non-thyrotoxic population of analogous composition. This discrepancy may be explained by the increased platelet adhesiveness demonstrated in thyrotoxic patients.

The decreased platelet adhesiveness in the hypothyroid state may explain why these patients escape the effects of the accompanying arterial disease until thyroxine is administered. The findings reported in this paper may indicate that increased platelet adhesiveness may be a risk factor beside many others in the pathogenesis of myocardial infarction.

ACKNOWLEDGEMENT

The investigation was supported by the Norwegian Council on Cardiovascular Diseases.

REFERENCES

- 1 Bennett, N. B., Ogston, C. M. & McAndrew, G. M. The thyroid and fibrinolysis. *Brit. med. J.* 4: 147 1967.

- 2 Borchgrevink, C. F. & Waaler, B. A. The secondary bleeding time: A new method for the differentiation of hemorrhagic diseases. *Acta med. scand.* 162: 361 1958.
- 3 Bennett, C. T. & Durbin, E. The signs and symptoms of heart changes in toxic goiter. *Amer. Heart J.* 8: 29 1932.
- 4 Burnstein, J., Lamber, B.-A. & Eritmaa, E. Myocardial infarction in thyrotoxicosis. *Acta med. scand.* 166: 379 1960.
- 5 Egeberg, O. Influence of thyroid function on the blood clotting system. *Scand. J. clin. Invest.* 15: 1 1963.
- 6 — Thyroid function and hemostasis. *Scand. J. clin. Lab. Invest.* 16: 511 1964.
- 7 Evans, G. & Irvine, W. T. Long-term arterial-graft patency in relation to platelet adhesiveness, biochemical factors and anticoagulant therapy. *Lancet* 2: 353 1966.
- 8 Grytting, O. & Salvesen, H. A. Thyrotoxicosis and myocardial infarction. *Acta med. scand.* 157: 169 1957.
- 9 Hellén, A. J. Platelet adhesiveness in von Willebrand's disease. A study with a new modification of the glass bead filter method. *Scand. J. Haemat.* 7: 374 1970.
- 10 Høme, R. Fibrinolytic activity and thyroid function. *Brit. med. J.* 1: 686 1965.
- 11 Jacobson, C. D. Proteolytic capacity in human plasma. Part I. Measurement of proteolytic activity in the presence of natural inhibitors and study of the inter-individual variations. *Scand. J. clin. Lab. Invest.* 21: 216, 1966.
- 12 Lamber, B.-A., Krivikangas, V., Peltkonen, R. & Vuopio, P. Thrombocytopenia and decreased life-span of thrombocytes in hyperthyroidism. *Ann. clin. Res.* 3: 98 1971.
- 13 Litman, D. S., Jeffers, W. A. & Rose, E. The infrequency of myocardial infarction in patients with thyrotoxicosis. *Amer. J. med. Sci.* 233: 10 1957.
- 14 Mørsted, J. F. & Pachatz, H. A. Factors influencing platelet function: Adhesion, release, and aggregation. *Pharmacol. Rev.* 22: 97 1970.
- 15 Myasnikov, A. L., Myasnikov, L. A. & Zaitsev, V. F. The influence of thyroid hormones on cholesterol metabolism in experimental atherosclerosis in rabbits. *J. Atheroscler. Res.* 3: 295 1963.
- 16 Segård, E., Solem, J. H. & Hellén, A. J. The adhesiveness of human blood platelets and thyroid function. *Acta endocr. (Kbh.) Suppl.* 177: 272, 1973.
- 17 Sotito, J. F., Moffat, D., Kolodzie, L. & Schaefer, R. Low serum platelet-8k activity (S. P. L. A.) and other hemostatic defects in hypothyroidism. 4th International congress on thromboses and hemostasis, Vienna 1973. Abstracts, p. 207. Grisel, Vienna 1973.
- 18 Vanhaebel, L., Neve, H., Chailly, H. & Bastien, P. A. Coronary-artery disease in hypothyroidism. *Lancet* 2: 800 1967.
- 19 Wayne, E. J. Clinical and metabolic studies in thyroid disease. *Brit. med. J.* 1: 78 1960.

Acta Chirurgica Scandinavica

Editor: O. Hultén

8 issues per volume. Free supplements, including free subscriptions to the *Scandinavian Journal of Plastic and Reconstructive Surgery* (without suppl.), the *Scandinavian Journal of Thoracic and Cardiovascular Surgery* (without suppl.) and the *Scandinavian Journal of Urology and Nephrology* (without suppl.)

Current volume 140/1974

Sw kr 250 per volume incl. postage

Acta Dermato-Venereologica

Editor: Nils Thyresson

6 issues per volume. Free supplements.

Current volume 54/1974

Sw kr 125 per volume incl. postage

Acta Medica Scandinavica

Editor: J. Waldenström

6 issues per volume. Free supplements.

Current volumes 197-198/1973

Sw cr 225 per annum (two volumes) incl. postage

Acta Obstetrica et Gynecologica Scandinavica

Editor: Axel Ingelman-Sundberg

4 issues per volume. Free supplements.

Current volume 53/1974

Sw kr 125 per volume, incl. postage

Acta Oto-Laryngologica

Editor: C. A. Hamberger

6 issues per volume. Free supplement

Current volumes 77-78/1974

Sw k 100 per volume. Two volumes per annum

Sw kr 200 incl. postage

Acta Paediatrica Scandinavica

Editor: R. Zetterström

11 issues per volume. Free supplement

Current volume 63/1974

Sw kr 150 per volume, incl. postage

International Journal of Fertility

Editor: S. J. Behrman

4 issues per volume.

Current volume 19/1974

Sw kr 110 per volume, incl. postage

Scandinavian Audiology

Editor: H. Birk Nielsen

4 issues per volume. Free supplements.

Current volume 3/1974

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Infectious Diseases

Editors: Justus Ström and Sten Winblad

4 issues per volume. Free supplements.

Current volume 6/1974

Sw kr 100 per volume incl. postage

Scandinavian Journal of Plastic and Reconstructive Surgery

Editor: Bengt Johansson

3 issues per volume. Free supplements.

Current volume 8/1974

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Psychology

Editor: Lars Kebabian

4 issues per volume.

Current volume 15/1974

Sw kr 78 per volume, incl. postage

Scandinavian Journal of Rehabilitation Medicine

Editor: Olle Håk

4 issues per volume. Free supplements.

Current volume 6/1974

Sw kr 75 per volume, incl. postage

Scandinavian Journal of Rheumatology

Editor: Velkko Laine

4 issues per volume. Free supplements.

Current volume 3/1974

Sw kr 90 per volume incl. postage

Scandinavian Journal of Social Medicine

Editor: Gunnar Inghe

3 issues per volume. Free supplements.

Current volume 2/1974

Sw kr 90 per volume incl. postage

Scandinavian Journal of Thoracic and Cardiovascular Surgery

Editor: Vilhelm Olov Björk

3 issues per volume. Free supplements.

Current volume 8/1974

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Urology and Nephrology

Editor: Gustav Gertz

3 issues per volume. Free supplements.

Current volume 8/1974

Sw kr 100 per volume, incl. postage

Uppsala Journal of Medical Sciences

Editor: Gunnar Ågren

3 issues per volume. Current volume 79/1974

Sw kr 60 per volume, incl. postage

Free inspection copies on request—write to

**The Almqvist & Wiksell Per
Box 62, S 101 20 St**

mpsn

THALASSAEMIA MINOR

Three Patients in two Norwegian Families

E. Jakobsen, H. C. Godal and P. Kierulf

*From the Hematological Research Laboratory, Medical Department D,
Ulrich's Hospital, Oslo, Norway*

Abstract Thalassaemia minor has been diagnosed in 11 members of two Norwegian families. Haematological data from 13 family members are reported. The diagnosis is based on estimation of Hb A₂ by polyacrylamide gel disc electrophoresis. All patients having high Hb A₂ levels had confirmatory evidence of thalassaemia minor (hypochromic anaemia, marked anisotom in size and shape of the red cells, target cells, basophilic stippling, decreased osmotic fragility, normal or high serum iron level). The majority of the patients had previously been treated with iron without effect.

The protein part of the haemoglobin molecule consists of two separate pairs of peptide chains. With each chain is associated a haeme group. The normal haemoglobins in adult life are Hb A and the minor component Hb A₂, which comprises about 2.5% of the total Hb. The globin chain composition of the two haemoglobins is $\alpha_2\beta_2$ and $\alpha_2\delta_2$ respectively (7, 8, 20).

The thalassaemia syndromes are a group of genetic disorders in which there is a defect in the rate of synthesis of one or more of the globin chains. In β -thalassaemia the most common form, the synthesis of β -chains is defective. The homozygous form thalassaemia major is usually a serious disease with grave haemolytic anaemia and death in infancy or childhood. In the heterozygous form thalassaemia minor or thalassaemia trait, however, the haematological findings are more moderate and the clinical symptoms mild if present at all. The reduced synthesis of β -chains is associated with an increase in the synthesis of δ -chains, consequently the most characteristic finding in thalassaemia minor is an increased level of Hb A₂.

The thalassaemia appear most frequently in the

Mediterranean region and the Far East. Outside these areas the disease is found in immigrant populations from countries where the disease is endemic. Thalassaemia minor is however occasionally found even in aboriginal North European people (1-4, 11, 13, 17, 18, 19). In the last few years the disease has been recognized also in Scandinavians (4, 17).

The present study reveals the haematological findings in 13 members of two Norwegian families in which thalassaemia minor was diagnosed in 11 of the family members.

MATERIAL

Of 25 living members of the two families, A and B, 13 were examined haematologically. The study includes 15 adults and 8 children (below 18 years). Fig. 1 shows the pedigrees of the two families. Both families came from the same area in the south-eastern part of Norway near Oslo (Nordre Romerik), but no relationship between the families was known. No ancestry from Mediterranean, African or Asian countries was suspected.

The probands in the two families were A-III-1 and B-II-2. The former, as referred because of refractory anaemia, in the latter suspicion of thalassaemia had already been raised and he was referred for Hb electrophoresis.

Of the 12 family members who proved to be affected by thalassaemia minor (vide infra) 6 adults (A-I-1, A-III-1, B-I-1, B-II-2, B-II-3, B-II-4) suffered from chronic anaemia and had repeatedly been treated with iron. Some also had been treated with vitamin B₁₂ or liver extracts. Two adults (A-II-3, A-II-1) had been healthy without previous diagnoses of anaemia. Four children appeared to have thalassaemia. Two of them (B-III-6, B-III-11) had periodically been treated with iron, one (B-III-1) had always looked pale whereas one (B-III-8) had given healthy impression. None of the affected family members had received blood transfu-

Table 1 Haematological findings in the family members examined (affected members given in *italics*)

Pedigree no.	Sex	Age (y)	Hb (g/100 ml)	RBC $\times 10^6$	MCH (pg)	MCV (μm^2)	MCHC (%)	Per 1 000 RBC		
								Retic.	Target cells	Basoph. stippling
A-I-1	♀	76	11.4	4.9	23	70	32	10	26	1
A-II-2	♀	52	15.0	4.7	32	91	34	1	0	0
A-II-3	♀	51	11.9	5.1	23	73	32	11	26	1
A-III-1	♀	27	11.6	5.0	23	70	33	12	44	2
A-III-2	♀	20	12.0	5.0	24	72	33	7	16	3
B-I-1	♀	67	10.3	4.1	25	72	34	8	8	1
B-I-2	♂	67	15.4	4.6	33	100	33	10	0	0
B-II-1	♀	46	13.5	4.1	34	100	34	6	0	0
B-II-2	♂	43	11.3	6.0	19	68	28	12	12	4
B-II-3	♀	42	10.9	5.3	21	64	32	15	12	2
B-II-4	♀	40	11.8	5.7	21	65	32	10	3	2
B-III-1	♂	22	17.4	5.2	33	96	37	5	0	0
B-III-2	♀	20	13.1	4.2	31	90	35	3	0	0
B-III-3	♂	18	15.2	4.7	32	90	36	9	0	0
B-III-4	♂	16	15.8	4.6	34	98	35	1	0	0
B-III-5	♂	18	18.4	5.5	34	97	35	14	0	0
B-III-6	♀	14	11.6	5.1	23	71	32	18	3	1
B-III-7	♂	15	19.2	5.1	30	88	34	9	0	0
B-III-8	♂	11	12.0	4.7	25	78	31	13	36	2
B-III-9	♂	8	15.0	5.2	29	84	35	7	0	0
B-III-10	♂	17	14.8	4.9	30	90	34	5	0	0
B-III-11	♀	13	12.0	5.5	22	53	41	10	4	6
B-III-12	♂	4	10.9	4.9	22	70	32	10	21	9

sions, except B-III-3 who had had transfusions during an operation. None gave a history of jaundice, gallstones, leg ulcer, and none of those examined clinically had any enlargement of the spleen.

METHODS

Routine haematological investigations followed standard techniques. Blood films for staining were made from blood anticoagulated with Na₂EDTA. Target cells were counted in part of the blood films where the cells were spread in an even, single layer and in which the normal red cells showed a clear central zone.

Osmotic fragility of the red cells was tested according to the method of Purpur et al. (16) as modified by Myhre (14).

Haemolysate for quantitation of Hb A₂ and Hb F was prepared according to Hutchinson (8) from freshly drawn blood with heparin as anticoagulant. 2-mercaptoethanol (final concentration 1:1000) was added to the water used for lysis of the washed packed cells. The final Hb concentration in the haemolysate was 12–13 g/100 ml.

Hb A₂ was estimated by polyacrylamide gel disc electrophoresis (Shandon apparatus) using 7% polyacrylamide gels prepared in vertical glass tubes 5×75 mm. A Tris/glycine buffer pH 8.9 (5160 g Tris, 348 g glycine, 1000 ml distilled water) was used. Before application the haemolysate was diluted 1:10 in a mixture

of Tris/glycine buffer (1 vol.) and 25% sucrose (1 vol.) and 10 μ l of this dilution (120–130 μ g Hb) was applied on each gel. A current of 2 mA per tube was used until the sample had entered the gel subsequently 4 mA per tube was applied. The electrophoresis was stopped when the main Hb band had migrated 26 mm from the top of the gel. The gels were scanned outside the glass tubes at 413 nm in Vitatron densitometer type TLD including an integrator.

Hb F was measured as alkali-resistant Hb by the method of Becke et al. (1) as modified by Weatherall and Clegg (20). Bone marrow studies were not included.

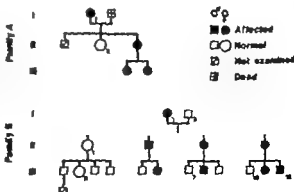


Fig. 1 Pedigrees of two Norwegian families with β -thalassaemia minor

m corp. paly NaCl)	Serum iron (μ g/ 100 ml)	TIBC (μ g/ 100 ml)	Serum h albumin (mg/ 100 ml)	Hapto- globin (mg Hb/ 100 ml)	Folic acid (pg/ml)		Serum vita- min B ₁₂ (pg/ml)	Hb F (% of total)	Hb A ₂ (% of total)
					Serum	Whole blood			
5	100	330	0.8	110				1.5	5.2
5	103	347	0.8	86				1.9	4
6	111	350	0.6	64				1.8	4.6
13	155	255	0.8	20	4.6	32	533	2.4	4.8
15	200	339	1.0	20	4.6	39	775	4	7.4
28	114	285	0.4	13		40		1	4.8
44	112	321	0.5	100				0.5	4.3
41	71	382	0.4	88				0.8	4.1
56	123	300	0.5	79				2.3	6.0
58	86	408	0.6	60	4	27	670	0.5	4.9
58	108	369	0.4	96	9	41	514	1.3	4.9
40	108	423	0.4	74				1.0	1.8
41	104	363	0.4	47				0.8	1.9
42	114	303	1.9	74				0.4	6
42	112	383	0.5	25				0.5	1.2
113	158	402	1	100				1.3	6
38	107	427	0.8	44	6.1	81	421	1.7	6.1
43	77	420	0.7	56				1.5	1.3
34	126	339	0.6	6				0	3.3
47	83	399	0.3	6				0.5	0.7
42	80	345	0.3	41				0.5	2.3
38	107	360	0.4	65	5.9	24	948	2.0	4
33	152	363	0.6	37				1.5	6.0

RESULTS

Table I summarizes the haematological data in the subjects examined. Based on the levels of Hb A₂ the family members could easily be divided into two groups (Fig. 2) and the diagnosis of thalassaemia minor could be stated by means of this estimation alone (Fig. 3). Twelve members were affected (males/females=3/9) and 11 were normal (males/females=8/3). Mean Hb A₂ value in the thalassaemia group was 5.1% of total Hb (range 3.3–7.4) and in the normal group 1.9% (range 0.7–4) (Table II). Those in the high Hb A₂ group presented outstanding morphological abnormalities of the red cells consistent with thalassaemia minor including anisocytosis, poikilocytosis, ovalocytosis and irregularly contracted cells, while none in the low Hb A₂ group revealed such findings. Consequently there was no overlap between affected and normals concerning the Hb A₂ level.

Mean Hb F was 1.8% of total Hb in the thalassaemia and 1.1% in the normal group. There was however a high degree of overlap between the two groups.

Mean Hb concentration was 11.5 g/100 ml (range 10.3–14.0) in the thalassaemia group and

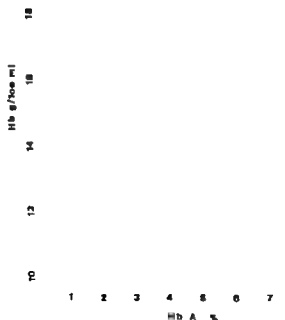


Fig. 2. Hb concentration and Hb A₂ level in the family members examined. Symbols as in Fig. 1.

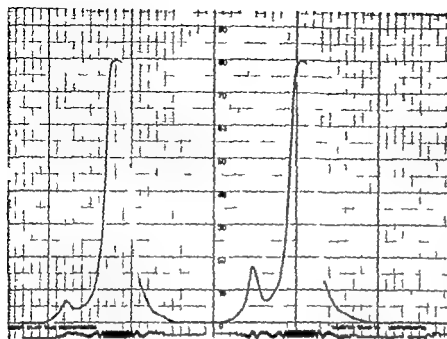


Fig. 3 Hb electrophoresis. Scanning profile of polyacrylamide gels (main peak = Hb A, minor peak = Hb A₂). Left: normal (B 117); right: β -thalassaemia minor (A 1117).

Table II Summarized data for all family members (mean values range within parentheses)

Haematological ups	β -thalassaemia minor			Normal		
	Total	Males	Females	Total	Males	Females
Hb (g/100 ml)	11.5 (10.3–12.0)	11.4 (10.9–12.0)	11.4 (10.3–12.0)	15.3 (13.1–18.4)	15.9 (14.8–18.4)	13.8 (13.1–15.0)
RBC $\times 10^6/\mu$ l	5.1 (4.1–6.0)	5.2 (4.9–6.0)	5.1 (4.1–5.7)	4.8 (4.0–5.5)	5.0 (4.6–5.5)	4.3 (4.0–4.7)
MCH (pg)	23 (19–25)	22 (19–25)	23 (21–25)	32 (29–34)	32 (29–34)	32 (31–34)
MCV (μ m ³)	69 (53–78)	70 (66–78)	68 (53–73)	93 (84–100)	93 (84–100)	94 (90–100)
MCHC (%)	33 (28–41)	31 (28–32)	33 (30–41)	35 (33–37)	35 (33–37)	34 (34–35)
Retic./1 000 RBC	11 (7–15)			6 (1–14)		
Ser Fe (μ g/100 ml)	125 (86–200)	133 (123–152)	122 (86–200)	99 (71–148)	102 (77–158)	93 (71–105)
TIBC (μ g/100 ml)	345 (255–477)	334 (300–363)	347 (255–427)	381 (303–463)	375 (303–423)	397 (347–463)
Bilirubin (mg/100 ml)	0.7 (0.4–1.0)			0.8 (0.3–2.1)		
Haptoglobin (mg Hb/100 ml)	58 (6–132)			63 (6–100)		
Hb F (% of total)	1.1 (0.4–3.8)			0.6 (0.3–1.0)		
Hb A ₂ (% of total)	5.1 (3.3–7.4)			1.9 (0.7–2.4)		

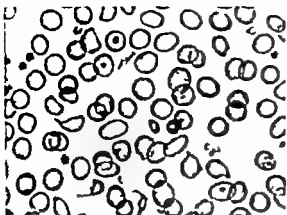


Fig. 4 Peripheral blood film of patient with β -thalassaemia minor (A-III-1) showing typical changes, including target cells.

15.3 g/100 ml in the normals. Within the thalassaemia group there was no correlation between Hb concentration and Hb A₂ level (Fig. 1).

Table II shows summarized additional data. RBC was moderately increased in the thalassaemia group. MCH and MCV were low in all affected members while the overlap of MCHC values between the thalassaemic and the normal members was considerable. Reticulocyte counts were within the normal range.

Target cells were easily detected and basophilic stippling was recognized in all subjects with thalassaemia minor (Fig. 4). Osmotic fragility was invariably decreased in this group. Fig. 5 shows a typical result of the osmotic fragility test in an affected member (B-III-1).

The serum iron level was high in 4 and within the normal range in the remainder of the thalassaemia group. None of the affected members had hyperbilirubinaemia, whereas 2 in the normal group (both 18-year-old males (B-III-3, B-III-5)) had increased serum bilirubin values without any laboratory evidence of haemolysis or liver disease. The findings may represent some type of inherited abnormality of bilirubin metabolism. The 4 subjects with high serum iron level (A-III-1, A-III-3, B-III-8, B-III-1) also had low haaptoglobin levels. One of them (A-III-1) has been followed for more than one year and the haaptoglobin values have invariably been low (10–20 mg Hb/100 ml). She also had moderately lowered folic acid values (Table I).

Correction of this deficit by folic acid did not, however, affect the Hb concentration.

DISCUSSION

Opinions differ as to the severity of anaemia in the heterozygous state of β thalassaemia. According to Weatherall and Clegg (70) the majority of β thalassaemia carriers are not anaemic. Several reports, however, have demonstrated a significant reduction of the Hb concentration in patent heterozygous for β -thalassaemia (3, 11, 17, 18). In the present study all affected family members were anaemic.

β -thalassaemia minor usually presents as a moderate hypochromic anaemia, and the red cell morphology may be similar to that of iron deficiency. Most frequently there is a slight compensatory erythrocytosis with moderately raised RBC. These features were all present in the affected members. An outstanding feature was also the abnormalities in red cell morphology consisting in marked variation in size and shape and easily detectable basophilic stippling and target cells. These morphological changes were apparently not correlated to the Hb concentration or the Hb A₂ level.

A haemolytic component is commonly partially responsible for the anaemia in thalassaemia minor (8). In our study only 4 of the 12 affected family members presented evidence of increased haemolysis (low haaptoglobin and high serum iron levels) but none had reticulocytosis or bilirubin concentration above normal or a history of jaundice. Characteristically resistance to osmotic lysis was constantly increased.

In contrast to the hypochromic anaemia the serum iron level is normal or raised in thalassaemia minor. In spite of this such patients are not infrequently treated mistakenly with iron (11, 17). Such treatment is unnecessary and potentially harmful. It should be noted, however, that these patients may develop iron deficiency from the same causes as others (dietary deficiency, chronic intestinal bleeding, pregnancy, etc.) and they consequently need iron.

Because of compensatory erythrocytosis patients with thalassaemia minor may suffer from folic acid deficiency, particularly in pregnancy, in which such deficiency is prone to be more marked in thalassaemia patients than in healthy pregnant

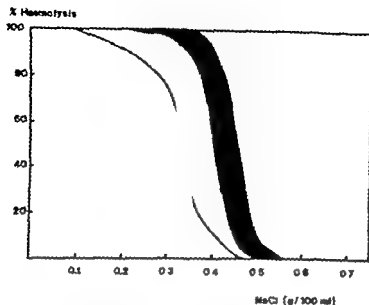


Fig. 5 Osmotic fragility test from a patient with β -thalassaemia minor (B-III 17). Dark area indicates the normal range

women. These patients should therefore be carefully supervised during this period with special attention to folate deficiency and evidence of a negative iron balance (9-15). Occasionally transfusions are necessary during pregnancy.

Thalassaemia minor should be suspected in patients with chronic hypochromic anaemia with normal or high serum iron level, particularly if there is a family history of chronic anaemia. The hereditary type of sideroblastic anaemia may be an early diagnosis. However, estimation of the

A_2 amount by Hb electrophoresis will confirm the diagnosis of thalassaemia minor. A raised Hb A_2 level can be considered virtually diagnostic of this disorder (6). If the thalassaemia is associated with iron deficiency, the Hb A_2 level may be depressed, and the diagnosis consequently missed (9-10). Another estimation should therefore be made after correction of the iron deficiency.

Hb electrophoresis may be performed in various media; most frequently starch gel and cellulose acetate are used. The polyacrylamide gel disc electrophoresis applied in this study appeared to be a suitable method. The Hb A_2 values obtained were in accordance with those found in other series (6, 11, 17, 18).

It may be suggested that the increasing element of immigrants and workers from the Mediterranean countries and Asia will focus the interest upon thalassaemia minor in the differential diagnosis of refractory anaemia. In North Europeans the disorder obviously is uncommon. The incidence is

not known, however, since no screening studies have been performed. As case reports are accumulating, the recognition of the disease will probably increase.

REFERENCES

1. Beike, K., Marti, H. R. & Schlicht, I. Estimation of small percentages of foetal haemoglobin. *Nature* 184: 1877-1959.
2. Buchanan, K. D., Kinloch, J. D., Hutchinson, H. E., Pinkerton, P. H. & Cassidy, P. Thalassaemia in Scots. *J. clin. Path.* 16: 596-1963.
3. Castaldi, G. & Zavagli, G. Anaemia in thalassaemia. *Lancet* 1254: 1972.
4. Evensen, S. A., Jeremic, M. & Hjort, P. F. Iron-resistant hypochromic anaemia in a Scandinavian family. Heterozygous β -thalassaemia. *Acta med. scand.* 186: 331-1969.
5. Freedman, W. L. Alpha and beta thalassaemia and pregnancy. *Can. Obstet. Gynec.* 12: 115-1969.
6. Gibbs, W. N. Hb A_2 and the diagnosis of β -thalassaemia trait. *W. Indian. med. J.* 18: 177-1969.
7. Huisman, H. F. Normal and abnormal human hemoglobins. *Advanc. clin. Chem.* 15: 149-1972.
8. Hutchinson, H. E. An introduction to the haemoglobinopathies and the methods used for their recognition, p. 88. Arnold, London, 1967.
9. Josephson, A. M., Mastri, M. S., Singer, L., Dworin, D. & Singer, K. Starch block electrophoretic studies of human hemoglobin solutions. II. Results in cord blood thalassaemia and other hemologic disorders. Comparison with Towhee electrophoresis. *Blood* 13: 343-1958.
10. Karanis, C., Lagos, P., Metastatos-Mavroudis, A. & Matsaniotis, N. Serum iron and unsaturated iron-binding capacity in the β -thalassaemia trait.

- Their relation to the levels of haemoglobins A, A₂ and F. *J. med. Genet.* 9: 154, 1972.
- 11 Knox-Macaulay H. H. K., Weatherall D. J., Clegg, J. B. & Pembrey M. E. Thalassaemia in the British. *British med. J.* 3: 150, 1973.
 - 12 Kunzel, H. ■ Ceppellini R., Müller-Eberhard, U. & Wolf J. Observation on the minor basic hemoglobin component in the blood of normal individuals and patients with thalassaemia. *J. clin. Invest.* 36: 1615, 1958.
 - 13 McCarthy G. M., Temperley I. J. & Path, M. C. Thalassaemia in an Irish family. *Irish J. med. Sci.* 1: 303, 1968.
 - 14 Myhre, E.. Erythrocyte fragility tests. *T noriske Lægeforen.* 80: 1045, 1960.
 - 15 Pakes, J. ■ Cooperberg A. A. & Gelfand, M. M. Studies on beta thalassaemia trait in pregnancy. *Amer. J. Obstet. Gynec.* 108: 1217, 1970.
 - 16 Parpart A. E. Lorenz, P. B. Parpart, E. R. Gregg, J. R. & Chase A. M.. The osmotic resistance (fragility) of human red cells. *J. clin. Invest.* 26: 636, 1947.
 - 17 Persson, S., Samuelson B. Sjölin, S. & Wallenius, G. β -thalassaemia in two Swedish families. *Scand. J. Haemat.* 4: 361, 1967.
 - 18 Nowicki L., Becker H. Behnken, L. Martin, H. & Sprenger A. Zur Diagnostik der Thalassaemia minor mit Bericht über eine weitere deutsche Thalassaemie Sippe. *Deutsch. med. Wochschr.* 97: 773, 1972.
 - 19 Vuopio P. Ikkala, E. & Nautinen, M.. β -thalassaemia minor in Finland. *Dooderich* 87: 1269, 1971.
 - 20 Weatherall D. J. & Clegg, J. B. The thalassaemia syndromes, 2nd ed. p. 374. Blackwell Oxford 1977.

CHANGES IN CERTAIN IRON METABOLISM VARIABLES AFTER A SINGLE BLOOD DONATION

Gödrun Liedén, Sörker Höglund and Lars Ehn

From the Blood Centre and the Department of Internal Medicine Regional Hospital, Luleå,
and the Section of Hematology Karolinska Hospital, Stockholm, Sweden

Abstract. Signs of iron deficiency have been studied after the first blood donation in 11 healthy men. Six were given 100 mg iron daily and five received placebo tablets. The total iron-binding capacity and iron absorption remained raised for more than 26 days, but had almost returned to the initial values after 70 days. A significant decrease in the stainable bone marrow iron could be shown in all subjects after 26 days, later some restoration was seen in subjects given iron supplements, but not in those given placebo. As the restoration takes are long, the interval after blood donation must be taken into account when judging iron metabolism variables in active donors.

Loss of blood causes changes in the haemoglobin, serum iron, stainable bone marrow iron, total iron-binding capacity and iron absorption from the intestine. It is known that most blood donors in Sweden show these signs of iron deficiency. In order to evaluate the effect of prophylactic measures the development of signs of iron deficiency needs to be elucidated. Blood donors have depleted iron stores and it is of interest to establish whether or not the iron stores are utilized in the generation of haemoglobin (10, 14, 20, 27).

MATERIAL AND METHODS

The series comprised 12 conscripts aged 19-22 years. Six were given 100 mg iron as ferrous carbonate (17). Six were given placebo tablets. All were found to be fit on examination before call-up and none gave history of haemorrhage or of previous blood donation. After the start of the study one man in the placebo group refused further laboratory investigations and was excluded.

Tablets were supplied for only three or four days at most. The men were asked at each visit whether they had taken all the earlier tablets, and they affirmed

this. Medication was stopped five days before each iron absorption test to avoid the masking effect of orally administered iron on the intestinal iron absorption (6, 9, 11, 16). Otherwise the tablets were given continuously throughout the study.

The haemoglobin (Hb) concentration was measured as oxyhaemoglobin in a cyanometer with filter 540 nm. Triplicate samples were taken with the subject standing. The serum iron and transferrin saturation (TIBC) and iron absorption were measured as described elsewhere (13, 15, 19, 28). Sedimentation rate was measured by the Westergren method. Bone marrow smears stained by the technique of Hansen and Wersfield (17).

Stainable bone marrow iron was semiquantitatively assessed in bone marrow squash preparations made as described by Hansen and Wersfield (17) and stained by the method of Rath and Fleish (21). Ten random smears of 15-20 bone marrow fragments on each of two slides from each femoral puncture were made on the same day. The slides were marked with slide numbers, making it impossible for the examiners to identify them. The histochemical grades were defined as described elsewhere (8, 19, 21, 23).

The initial values for all variables to be studied were measured, all subjects gave 420 ml blood and the iron studies were repeated during the following 14 weeks. The Hb concentration was measured daily during the first week, and subsequently twice weekly. Other variables were measured on an average 11.4 days (range 8-14), 26.3 days (range 22-31) and 70.3 days (range 60-82) after the blood donation. The last diagnostic iron absorption test was done only in the iron group.

RESULTS

In the iron group the greatest fall in Hb was noted after three days and the initial Hb concentration was regained after 10 days (Fig. 1). The serum iron values showed considerable individual variations and no conclusions can therefore be drawn.

Table 1 Iron group Initial values for serum iron TIBC 70 days after a first blood donation

Tests of significance (one-sided t -test) on mean changes for the 11th and 26th day measurements: serum iron not significant, TIBC $p < 0.02$, stainable bone marrow iron $p < 0.02$, iron absorption $p < 0.02$

Subj. no	Serum iron ($\mu\text{g}/100 \text{ ml}$)				TIBC ($\mu\text{g}/100 \text{ ml}$)				Stainable bone marrow iron (histochemical grades)				Diagnostic iron absorption test (%)			
	Initial value	Change at		70 d	Initial value	Change at		70 d	Initial value	Change at		70 d	Initial value	Change at		70 d
		11 d	% d			11 d	% d			11 d	% d			11 d	% d	
1	171	-78	-84	+2	378	+65	+75	+3	4	-1	-2	-0.5	48.2	-2.4	+76.2	-14.7
2	75	+9	+49	-17	403	+56	+31	+13	4	-1	-0.5	-0.5	35.1	+59.9	+33.8	-1.8
3	87	+8	+87	+51	460	+59	+35	+2	3.4	0	0	-0.5	10.6	+55.3	+5.0	+31.9
4	123	-35	-35	-1	397	-	+25	+5	4	-0.5	-2	-1	32.6	+43.0	-70.8	-5.1
5	95	-17	-17	-3	369	+40	+40	+3	4	0	0	-0.5	43.7	+29.3	(-18.4)	+14.1
6	134	18	-24	+24	326	+46	-10	-16	2	-0.5	-1.5	-0.5	35.9	+21.0	+11.4	+4.3
Mean																
\pm S.E.M.	114 \pm 15	-15	-1	+10	372 \pm 11	+53	+35	+8	3.6 \pm 0.8	-0.5	-1.2	-0.7	34.4 \pm 5.3	+31.1	+20.5	+4.8

Table 11 Placebo group Initial values for serum iron TIBC stainable bone marrow iron and diagnostic iron absorption test and changes at 11, 26 and 70 days after a first blood donation

Test of significance (one-sided sign test) on mean changes for the 11th and 26th day measurements: serum iron not significant, TIBC p near 0.05, stainable bone marrow iron p near 0.06, iron absorption not significant

Subj. no	Serum iron ($\mu\text{g}/100 \text{ ml}$)				TIBC ($\mu\text{g}/100 \text{ ml}$)				Stainable bone marrow iron (histochemical grades)				Diagnostic iron absorption test (%)			
	Initial value	Change at		26 d	Initial value	Change at		26 d	Initial value	Change at		70 d	Initial value	Change at		26 d
		11 d	26 d			11 d	26 d			11 d	26 d					
7	109	-12	-27		372	0	+37		5	0	-2	-1.5	36.6	(-34.1)	-6.9	
8	102	+86	+86		375	+71	+71		3.4	-	-0.5	-1.5	35.0	-	+25.4	
9	170	-108	-6		378	+13	+62		3.4	0	0	-1.5	30.7	-8.7	+7.1	
10	172	+19	-4		381	+59	+37		3	-2	-2	-2	66.6	+8.8	+11.5	
11	205	-55	-55		350	-	+22		3.4	-0.5	-1.5	-2	34.8	+27.9	+12.7	
Mean																
\pm S.E.M.	144 \pm 19	-34	-11		373 \pm 7	+24	+46		3.7 \pm 0.8	-0.6	-1.2	-1.7	40.7 \pm 6.5	+8.6	+10.0	

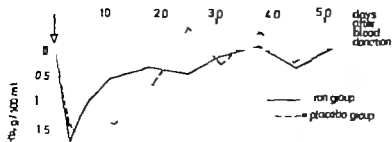


Fig 1 Mean decrease in Hb concentration after first blood donation in subjects given 100 mg iron or placebo tablets once daily. Values from two samplings are combined (thus, day 4+5 days 6+7 15 weeks, etc).

The TIBC increased significantly the mean increase after 11 days being $53 \mu\text{g}/100 \text{ ml}$ and after 26 days $35 \mu\text{g}/100 \text{ ml}$. After 70 days the values had returned to the initial levels in most subjects (Table I).

The diagnostic iron absorption test showed a statistically significant change (mean increase 31% after 11 days and 20.5% after 26 days). One subject (no. 5) was not strictly fasting when the 26th day test dose was given, and this value was therefore omitted. At the fourth measurement, 70 days after the blood donation the iron absorption had returned to approximately the initial level in most subjects (mean increase +4.8% Table I).

The amount of stainable bone marrow iron showed a slight fall after only 11 days after 26 days the mean reduction was 1 histochemical grade which is statistically significant. A return towards initial levels then took place but no subject showed a fully restored bone marrow iron level at the time of the last examination 70 days after the blood donation.

In the placebo group the Hb was lowest five days after the blood donation, and had regained its initial concentration after 25 days (Fig 1). After 26 days a statistically significant TIBC increase (mean $46 \mu\text{g}/100 \text{ ml}$) was seen and all subjects but one showed increased iron absorption as compared to the initial values (Table II).

The amount of stainable bone marrow iron was reduced in all subjects but one 26 days after the blood donation. In contrast to the iron group the decrease persisted the difference being statistically significant at the last examination 70 days after the blood donation.

With one exception, when poor quality smeared made counting impossible sideroblasts constituted 13% or more of the erythroblasts in all bone marrow samples even in the placebo group as early as 11 days after the blood donation.

DISCUSSION

Iron stores and haemoglobin regeneration. Full restitution of the Hb concentration took 25–10 days which is in accordance with the findings of Wadsworth (25) and Alstead (1). In persons who have given blood repeatedly (4 5 7 22 24 26) and who may well have had depleted iron stores, the Hb regeneration time is longer. It would therefore appear that storage iron can be used for Hb formation. This is also suggested by the reduction in the stainable bone marrow iron found in the present study after a blood loss as small as 50 ml (blood donation 420 ml plus two 50 ml samples for serum iron and TIBC estimation).

Iron for Hb regeneration after a blood loss may also be derived from absorbed iron (14) but in our iron group the Hb restitution time is so shorter than among the men receiving placebo. Moreover the decrease in storage iron during the first 26 days after a blood donation, i.e. the time when Hb is reforming, was similar in both groups. This again suggests that, under the conditions of the study the main source of iron for Hb regeneration was the iron stores even when supplementary iron was given by mouth.

Persistence of iron deficiency signs. Increased absorption was demonstrable for more than 26 days which is a little longer than previous studies have indicated (3 27). The TIBC increase persisted for more than 26 days which is also a little longer than previously shown (18).

Signs of iron deficiency thus remain for a considerable time after single blood donation. If donors give blood every two months, which is not unusual in Sweden they can be expected to show signs of iron deficiency for most of that time.

ACKNOWLEDGEMENT

The study was supported by a grant from AB Astra, Södertälje.

REFERENCES

- Altstead S. Rate of blood-regeneration after haemorrhage. *Lancet* 1: 424 1943
- Babson, A. & Kleinman N. A source of error in an auto-analyzer determination of serum iron. *Clin. Chem.* 13: 163 1967
- Bothwell, T. H. Pirzio-Biroli, G. & Finch C. A. Iron absorption. I. Factors influencing absorption. *J. Lab. clin. Med.* 51: 24 1958
- Brewer H. F. ref. in Norberg, B. & Ramgren, O. Hämoglobin- serumjärn och serumproteiner hos blodgivare. *Nord. Med.* 56: 1649 1956
- Cole B. T. Penrod K. E. & Hall, F. G. The hematocrit and hemoglobin response of blood donors. *J. Aviat. Med.* 24: 227 1953
- Conrad, M. E. & Crosby W. H. Intestinal mucosal mechanisms controlling iron absorption. *Blood* 22: 406 1963
- Fowler W. M. & Barer A. P. Rate of hemoglobin regeneration in blood donors. *J. Amer. med. Ass.* 118: 421 1942
- Gale E. Torrance J. & Bothwell T. The quantitative estimation of total iron stores in human bone marrow. *J. clin. Invest.* 42: 1076 1963
- Granick, S. Increase of the protein apoferritin in the gastrointestinal mucosa as a direct response to iron feeding. The function of ferritin in the regulation of iron absorption. *J. biol. Chem.* 164: 737 1946
- Iron metabolism and hemochromatosis. *Bull. N. Y. Acad. Med.* 25: 403 1949
- Hahn H. F. Bide, W. F. Ross J. F. Balder W. M. & Whipple G. H. Radioactive iron absorption by gastro-intestinal tract. *J. exp. Med.* 78: 169 1943
- Hansen, H. A. & Weisfeld, A. Hemosiderin estimations and sideroblast counts in the differential diagnosis of iron deficiency and other anemias. *Acta. med. scand.* 163: 333 1959
- Henry R. J. Sobel, Ch. & Chiamori, N. On the determination of serum iron and iron binding capacity. *Clin. chim. Acta* 3: 523 1958
- Höllman R. H. & Henderson, P. A. Control of marrow production by the level of iron supply. *J. clin. Invest.* 48: 434 1969
- Höglund, S. Iron absorption in apparently healthy men and women. *Acta med. scand.* 186: 487 1969
- Transitory effect of oral administration of iron on iron absorption. *Blood* 34: 505 1969
- Höglund S. Ehn L. & Essander H. Resorptionen av järn från ferrokarbonat. *Opusc. med.* 18: 212, 1973
- Laurell C. B. Studies on the transportation and metabolism of iron in the body. *Acta physiol. scand., Suppl.* 46, 1947
- Liedén G., Höglund, S. & Ehn, L. Iron supplement to blood donors. II Effect of continuous iron supply. *Acta med. scand.* 197: 37 1975
- Pollycoate M. & Mortimer R. The quantitative determination of iron kinetics and hemoglobin synthesis in human subjects. *J. clin. Invest.* 40: 753 1961
- Pritchard J. A. & Scott D. E. Iron demands during pregnancy. In: Iron deficiency (ed. L. Hallberg, H.-G. Harwerth & A. Vassotti) p. 173. Academic Press, London and New York 1970
- Ramgren O. & Tengberg, J. E. Profylaktisk järntillskott till blodgivare. *Nord. Med.* 63: 488 1961
- Rath C. E. & Finch, C. A. Sternal marrow hemosiderin. *J. Lab. clin. Med.* 33: 81 1948
- Sentry A. C. The response of blood donors to iron. *Amer. J. med. Sci.* 201: 790 1941
- Wadsworth O. R. Recovery from acute haemorrhage in normal men and women. *J. Physiol.* 129: 583 1953
- Walsh R. J. & Sewell A. K. Some effects of blood loss on healthy males. *Med. J. Aust.* 1: 73 1946
- Weinstaub, L. R. Conrad M. E. & Crosby W. H. The significance of iron turnover in the control of iron absorption. *Blood* 24: 19 1964
- Young, D. S. & Hicks J. M. Method for the automatic determination of serum iron. *J. clin. Path.* 18: 98 1965

IRON SUPPLEMENT TO BLOOD DONORS

1 Trials with Intermittent Iron Supply

Gudrun Liedén

From the Blood Centre and the Department of Internal Medicine Regional Hospital, Linköping, Sweden

Abstract A series of 58 male blood donors has been studied with regard to stainable bone marrow iron, desferrioxamine test, sideroblasts, total iron-binding capacity, serum iron and haemoglobin values. With one conventional blood donation every second month the storage iron was found to be significantly decreased after four blood donations both when placebo and oral ferrous iron in doses of up to 2000 mg were given over a period of two weeks after each blood-letting. The iron state in donors given 2000 mg was superior to that in donors given 1000 mg, and better in the latter group than when placebo was given. A moderate increase in the total iron-binding capacity could be discerned in subjects treated with placebo or only 1000 mg iron after each donation, but no changes in serum iron or haemoglobin were noted. A smaller series of 13 donors was also investigated after six donations and was found to show essentially the same pattern in the iron state variables as after four donations. When the interval between donations was four months and 2000 mg ferrous iron was given over two weeks after each donation, all variables including the amount of bone marrow iron and the desferrioxamine test remained unchanged in ten subjects after four donations. Two subjects showed a moderate decrease in the stainable bone marrow iron, but it did not disappear completely.

Blood donation implies loss of iron from the donor. Although blood donors relatively seldom become patently anaemic (2, 3, 13, 14, 20), they are often found to have markedly reduced storage iron (5, 9, 19). Reduced iron stores and increased total iron-binding capacity but no other signs of iron deficiency were found in 60 regular male blood donors bled five times yearly and supplied with 600 mg ferrous fumarate after each blood donation (15). The present investigation was undertaken in order to find a model for iron supply that will compensate also for the loss of storage iron.

Two different blood donation schedules were studied in one (study A) the donation interval was two months in the other (study B) four months. In study A the donors received 2000 or 1000 mg iron or placebo after each donation and a control group was included. In study B all subjects donated blood and were given 000 mg iron after each donation.

METHODS

Haemoglobin (Hb) concentration was measured in study A as oxyhaemoglobin, in study B as cyanmethaemoglobin. In both studies commercial Hb solution (Acoglobin Ortho) was used for standardization. Duplicate samples were taken from venous blood drawn under light stress after the subjects had been lying down about 10 min (initial and final examinations) or at the end of a blood donation.

Samples for serum iron and total iron-binding capacity (TIBC) were taken simultaneously with the Hb samples. The subjects were not fasting, but all sampling was done at the same time of day (1-3 p.m.). The analyses were performed in a Technicon Auto-Analyzer using the methods of Young and Hicks (21) and Henry et al. (11). In study B the analyses were modified as described by Babson and Kienast (1).

Stainable bone marrow iron was evaluated semiquantitatively on bone marrow squash preparations stained as described by Rath and Fisch (17). Two examiners made separate assessments of two coded slides from each sternal puncture using a 6-grade scale 0-5 (16). In certain cases half-grades were employed, i.e. when both examiners had difficulties in placing their estimates within one of two adjacent grades or when the difference was 1 histochemical grade. The correlation between the gradings was good, in only two pairs of slides did the differences exceed 1 histochemical grade.

Sideroblasts were counted in bone marrow smears stained as described by Hansen and Wenzel (8).

Table I Study A. Peripheral blood variables
Initial values (mean \pm S.D.)

Group		Hb (g/100 ml)	Serum iron (μ g/100 ml)	TIBC (μ g/100 ml)
Controls	13	14.4 \pm 0.73	96 \pm 19	—
2 000 mg	12	14.5 \pm 0.42	106 \pm 18	385 \pm 21
1 000 mg	12	14.4 \pm 0.73	99 \pm 27	399 \pm 28
Placebo	18	14.6 \pm 0.90	103 \pm 22	373 \pm 32

Desferrioxamine test (DF test) was performed as described by Hallberg and Hedenberg (6) with i. injection of 10 mg desferrioxamine/kg b.wt. Basal excretion was measured in sample obtained immediately before the injection, and urine was collected for 16 hours.

STUDY A

Blood Donations Every Second Month

The study embraces four groups of subjects three of which served as blood donors, each individual having been supplied with 2 000 or 1 000 mg iron or placebo after each donation, the fourth group served as controls. The study has been published earlier in Swedish (4) and a summary is given here.

All subjects were young conscripts at the same regiment. All were healthy with no past history of haematological, gastrointestinal or renal disorder. None had had previous haemorrhage or had served as a blood donor.

Eighty volunteers were allocated at random to one of four groups. During the course of the study 22 of subjects were transferred to other regiments and

did not fulfil the experiment. Three members of the 2 000 mg group returned at the end of their period of service; they were examined after six but not after four blood donations. No other subjects withdrew from the

investigation. The mean age of the 58 subjects completing the experiment was 20 years (range 18–23).

Design of the study The blood donors gave blood every second month. Hb, serum iron and TIBC determinations were done before the first and then at the end of every donation. The blood loss on each occasion including samples was 450 ml. Hb, serum iron and TIBC measurements, bone marrow examination, and DF test were done in all subjects after four donations. Four or five members of each group were re-examined after six donations; three members of the 2 000 mg group were examined only after six donations.

The interval between the fourth donation and the final investigation varied in the 000 mg group between 7 and 30 days (mean 19) in the 1 000 mg group between 5 and 90 days (mean 37) and in the placebo group between 2 and 93 days (mean 42). The interval after the sixth donation, similar in all groups, was 3–18 days (mean 9.5).

After each donation two groups were given iron tablets (Ferrosyn S[®] containing 37 mg iron as ferrous succinate and 0.11 g succinic acid). The 2 000 mg group received two tablets twice daily for two weeks and the 1 000 mg group one tablet twice daily. The placebo group received two placebo tablets twice daily. The subjects were questioned about tablet-taking at every blood donation and at the final investigation. Some admitted not taking every tablet but not more than 10 tablets were "forgotten" by any subjects during the whole period.

The control group gave no blood and no iron was administered. Hb, serum iron and TIBC measurements were done at times corresponding to the donor groups' second donation.

RESULTS

The initial values for Hb, serum iron and TIBC are given in Table I. Owing to a mishap TIBC was not measured in the control group.

Table II Study A. Iron state at and after the fourth blood donation. Concerning Hb, serum iron and TIBC differences in individual values between the fourth and first donations are given.

Group		Difference in mean values between the fourth and first donations			After the fourth donation		
		Hb (g/100 ml)	Serum iron (μ g/100 ml)	TIBC (μ g/100 ml)	Sideroblasts, subjects with subnormal counts* (% of whole group)	Stainable bone marrow iron (histochemical grades, median value)	DF test* (mean \pm S.D.) (μ g/kg b.wt./16 h)
Controls	13	+0.29 NS	+28 NS	-24 NS	0	4	7.56 \pm 1.55
2 000 mg	12	+0.59 NS	+29 NS	+17 NS	23	2.5	6.76 \pm 1.95
1 000 mg	12	+0.30 NS	+9 NS	+36	42	1	5.30 \pm 1.59
Placebo	18	+0.35 NS	-8 NS	+30 ^a	43 ^a		4.82 \pm 1.09

NS = insignificant difference ($p > 0.05$), $0.01 < p < 0.05$ 12.5% or less. One subject had given blood less than ten days before sternal puncture.

Student's *t*-test: controls compared with 2 000 mg group NS, 1 000 mg group $0.001 < p < 0.01$, placebo group $p < 0.001$.

Table III. Study A Iron state at and after the sixth blood donation Concerning Hb serum iron and TIBC differences in individual value between the sixth and first donations are given

Group		Difference in mean values between the sixth and first donations			After the sixth donation		
		Hb (g/100 ml)	Serum iron (μ g/100 ml)	TIBC (μ g/100 ml)	Sideroblasts, subjects with subnormal counts* (% of whole group)	Stainable bone marrow iron (histochemical grades, median value)	DF test [†] (mean \pm S.D.) (μ g/kg b.w./16 h)
Controls	5	+0.40 NS	+47 NS	+1 NS	0	4	7.61 \pm 1.7
000 mg	7	+0.72 NS	+17 NS	+37 NS	29	0.5	6.27 \pm 1.22
1000 mg	4	+0.90 NS	-21 NS	+42 NS	79 [‡]	0	4.37 \pm 1.53
Placebo	5	+0.54 NS	+14 NS	+38 NS	80 [‡]	0	4.94 \pm 1.61

NS=insignificant difference ($p > 0.05$).

[†]12% or less. Two subjects had given blood less than ten days before sternal puncture. One subject had given blood less than ten days before sternal puncture.

[‡]Student *t*-test: controls compared with 000 mg group NS 1000 mg group NS placebo group 0.01 $< p < 0.05$

The iron state after four and six blood donations is summarized in Tables II-IV. Both after four and six blood donations less stainable bone marrow iron was found in all donor groups than in the controls and less in the 1000 mg and placebo groups than in the 000 mg group (Table IV). The difference is statistically highly significant.

The DF test values were higher in the controls than in the donors and the smaller the dose of prophylactic iron the lower the iron excretion. After six donations the mean DF value in the 1000 mg group is numerically lower than after four donations, but the difference in comparison

with the controls does not reach statistical significance probably due to the smaller number of subjects investigated after six donations.

Subnormal sideroblast counts were found in all donor groups the 1000 mg and placebo groups showing the largest number of subnormal counts. In four subjects the subnormal count may be due to the short postdonation interval (% 16).

Statistically significant increases in mean TIBC were found in the 1000 mg and placebo groups at the fourth donation compared to the first. The increase at the sixth donation was numerically equal and was at this point also seen in the 000 mg group. The differences at the sixth dona-

Table IV. Study A Classification of individuals in each group with regard to the amount of stainable bone marrow iron

Stainable bone marrow iron (histochemical grade)	After the fourth donation				After the sixth donation			
	Controls (n=13)	000 mg (n=12)	1000 mg (n=17)	Placebo (n=18)	Control (n=5)	000 mg (n=7)	1000 mg (n=4)	Placebo (n=5)
4-5	1							
4	8	2	1		3			
3-4	3		1					
3	1	4	1	1				
2-3								
2		1	1	4		1		1
1-								
1		1	7	7			1	1
0-1				1			1	
0			1					3

Mann-Whitney *U*-test (18) after the fourth donation controls compared with 000 mg group $p < 0.002$, 1000 mg group $p < 0.001$, placebo group $p < 0.001$. After the sixth donation controls compared with 000 mg group $p < 0.001$, 1000 mg group $p < 0.01$, placebo group $p < 0.01$.

Table V Study B Mean Hb serum iron TIBC and DF test values before the first and 15 weeks after the fourth donation (mean \pm S.D.)

	Hb (g/100 ml)	Serum iron (μ g/100 ml)	TIBC (μ g/100 ml)	DF test (μ g/kg b wt./16 h)
Initially	14.78 \pm 1.14	111 \pm 27	318 \pm 39	7.25 \pm 1.79
After 4th donation	14.88 \pm 0.92	119 \pm 35	323 \pm 40	7.09 \pm 1.72
Difference	+0.10 NS	+8 NS	+5 NS	-0.16 NS

Student's *t*-test NS=not significant.

tion do not reach statistical significance probably due to the small number of subjects.

Although initial values for DF test and stainable bone marrow iron are lacking comparison with the control group makes it seem very likely that a decrease in storage iron had already taken place after four blood donations in all donors. Not even doses of 2000 mg ferrous iron after each blood donation seem to have been enough to maintain the iron stores.

STUDY B

Blood Donations Every Fourth Month

The subjects were 15 men offering blood at the Blood Centre. One had had a peptic ulcer 15 years previously but no others gave any history of bleeding or had given blood. All claimed to be fit and not to have

Terred from any haematological or renal disorder examination before registration no abnormalities found. During the study one subject settled elsewhere and another refused further venal punctures. Thirteen subjects aged 18-45 years (mean 37) completed the study.

Design of the study Before the first blood donation Hb, serum iron and TIBC determinations, assessment of stainable bone marrow iron sideroblast counts and DF test were performed.

Four blood donations were made at mean intervals of 15.9 weeks (range 15.7-17.7). After each donation the subjects were given iron tablets (Ferrosyn S[®] containing 37 mg iron as ferrous succinate and 0.11 g succinic acid) two tablets twice daily for 14 days i.e. 2000 mg after each blood-letting. One week before each donation the iron state was checked by reexamination of all variables in part of the series (8 subjects before the second donation 7 before the third and 5 before the fourth). At 15 weeks after the fourth blood donation all subjects were reexamined. All were thus investigated at least 3 times, and seven 4 times, during the study.

RESULTS

The mean Hb serum iron TIBC and DF test values found initially and 15 weeks after the fourth donation are shown in Table V. The differences between the initial and final values are slight and statistically insignificant.

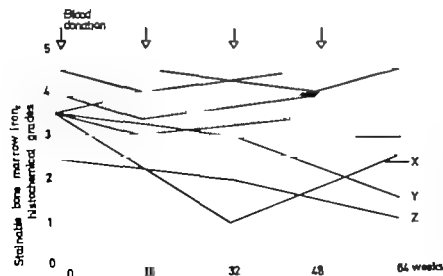


Fig. 1 Study B. Semi-quantitative assessment of the stainable bone marrow iron in 11 subjects all of whom were examined before the first and 15 weeks after the fourth blood donation. Subjects X, Y and Z are considered separately under Results.

The findings concerning stainable bone marrow iron are presented individually in Fig. 1. In 10 subjects it has remained virtually unchanged, the slight changes not exceeding 1 histochemical grade. In some of the 10 subjects are probably due to errors of the method.

Subjects X, Y and Z call for comment. In X a marked decrease in bone marrow iron had taken place before the third donation. This man conceded that he had taken only a few tablets prescribed. Before the final investigation he took all the remaining tablets, the bone marrow iron increased but did not regain the initial level. In Y and Z a moderate gradual decrease is seen and the difference between the initial and final figures exceeds 1 histochemical grade. Both subjects asserted that they had in fact taken all their tablets.

Sideroblast counts were normal ($\geq 13\%$) in every bone marrow smear from all subjects.

DISCUSSION

Study A shows that the storage iron decreases rapidly when blood donors are bled every second month and when iron is not given. After four donations one third of the placebo group had no stainable bone marrow iron, all subjects but one had less bone marrow iron than the control group, the mean DF test value was significantly lower than in the controls, and 43% had subnormal sideroblast counts.

If oral iron was given, as in the 1000 and 2000 mg groups, more storage iron remained, but the amount was significantly less than in the control group. However the results are influenced by the interval since the latest blood donation as a decrease in the amount of stainable bone marrow iron is demonstrable even after a single blood donation (16). Comparison with the control group ought ideally to be made using samples influenced as little as possible by the latest blood donation, e.g. samples taken immediately before or at the time of donation. The mean decrease after a single donation has been shown to be 1 histochemical grade after 26 days and about $\frac{1}{2}$ grade after 11 days. When the amount of bone marrow iron in each subject in the 2000 mg group is corrected by +0.5 (postdonation interval 7–18 days) or by +1.0 (interval 19–30 days) the estimations represent rough-

ly what would be expected at the preceding donation. Comparison between the bone marrow iron in the 2000 mg group (corrected as described) and the controls nevertheless discloses a statistically significant difference at the 0.05 level at the fourth, and at the 0.005 level at the sixth donation.

The DF test, which has been found to have a high correlation to storage iron as determined by chemical methods (7), parallels the results of bone marrow iron examination in this study: all donor groups excreted less iron than the control group, the difference being statistically significant for the 1000 mg and the placebo groups.

The dissimilarity in postdonation intervals in the donor groups may bias a comparison between them somewhat, but it seems likely that in comparison to the 1000 mg and placebo groups the 2000 mg group showed a superior iron state at all events after four donations.

Essentially the same findings as in the 1000 mg and placebo groups after four donations (reduced iron stores and raised TIBC but no other signs of iron deficiency) were obtained in regular donors who had given blood 15 times and more than 50 times, all of them at a rate of five times yearly and all receiving an iron supplement of 600 mg after each donation (15). This stage of iron deficiency without anaemia thus develops rapidly in donors bled every second month, but seems not to be aggravated by further blood donation. The reason for this is probably the increased iron absorption that takes place in subjects with diminished iron stores (10, 12).

If the interval between donations is increased to four months and 2000 mg iron is given as in study B all the variables studied including bone marrow iron and DF test remain essentially constant. This indicates that 2000 mg iron by mouth during the two weeks following each blood donation in addition to the iron in the food will be enough to compensate fully for the iron loss due to conventional blood donation in most adult men if the interval between donations is four months. The subject who did not take all his tablets demonstrates the necessity of supplementary iron in this regime. Subjects Y and Z who showed a moderate fall in storage iron, indicate that the chosen interval of 16 weeks is not too long as a general recommendation if the iron stores in the donors should remain unchanged.

ACKNOWLEDGEMENTS

Study A was supported by AB Hålsjö Mölndal and study B by the Medical Research Fund County of Östergötland.

REFERENCES

- 1 Babson, A. & Kleinman, N. A source of error in an AutoAnalyzer determination of serum iron. *Clin. Chem.* 13 163 1967
- 2 Debing H & Sachs, V. Wie belastend ist regelmäßiges Blutspenden für den Eisenhaushalt? *Münch. med. Wochr.* 114 1261 1972.
- 3 Dybkiær E. Ansættelse hos bloddonorer. *Ugeskr. Læg.* 127 1163 1963
- 4 Elm L, Ljeden, G & Ockfeld, C. O Järnbehov hos blodgivare. *Läkartidningen* 63 687 1968.
- 5 Fiedling J, Karabus C. & Brunsström G M. Storage iron depletion in male blood donors: its significance for iron status in women. *J. clin. Path.* 21 402, 1968
- 6 Hallberg L. & Hedenberg L. The effect of desferrioxamine on iron metabolism in man. *Scand. J. Haemat.* 2 67 1965
- 7 Hallberg, L., Hedenberg, L. & Wessaf M A. Liver iron and desferrioxamine-induced urinary iron excretion. *Scand. J. Haemat.* 3 85 1966.
- 8 Hansen H A. & Weisfeld A. Hemosiderin estimations and sideroblast counts in the differential diagnosis of iron deficiency and other anemias. *Acta med. scand.* 165 333 1959
- 9 Hausmann K. & Kuse R. ref in Heinrich, H C. Prälatente latente und manifeste Eisenmangelanämie bei Blutspendern. *Münch. med. Wochr.* 110: 1843 1968
- 10 Hausmann K, Kuse R, Sonnenberg, O W, Barrels H & H Isrich H C. Interrelations between iron stores, general factors and test results in iron absorption. *Acta haemat.* (Basel) 42: 193 1969
- 11 Henry R J, Sobel Ch. & Chierotti N. On the determination of serum iron and iron binding capacity. *Clin. chim. Acta* 3 523 1958
- 12 Höglund S, Elm L. & Ljeden G. Studies in iron absorption. VII Iron deficiency in young men. *Acta haemat.* (Basel) 44 193 1970
- 13 Knudsen, E. E. & Abrahams J. Haemoglobinvärdering hos bloddonorer. *Ugeskr. Læg.* 130: 1609 1968
- 14 Kwa, S B. The effect of repeated blood donation on the haemoglobin haematocrit and serum iron values of regular blood donors. *Singapore med. J.* 10: 139 1969
- 15 Ljeden G. Iron taste in regular blood donors. *Scand. J. Haemat.* 11 342, 1973
- 16 Ljeden G, Höglund S & Elm L. Changes in certain iron metabolism variables after a single blood donation. *Acta med. scand.* 197 27 1975
- 17 Rath C. E. & Flach, C A. Sternal marrow hemosiderin. *J. Lab. clin. Med.* 33 81 1948
- 18 Siegel S. Nonparametric statistics. McGraw-Hill New York Toronto and London 1956.
- 19 Weisfeld A. Storage iron in man. *Acta med. scand. Suppl.* 427 1964
- 20 Williamson J L. Haemoglobin levels in blood donor volunteers. *J. Irish med. Ass.* 61 206, 1968
- 21 Young, D S & Hicks J M. Method for the automatic determination of serum iron. *J. clin. Path.* 18 98 1965

IRON SUPPLEMENT TO BLOOD DONORS

II. Effect of Continuous Iron Supply

Gudrun Ljeden, Sverker Höglund and Lars Ehn

*From the Blood Centre and the Department of Medicine, Regional Hospital, Linköping
and the Section of Haematology, Karolinska Hospital, Stockholm, Sweden*

Abstract. Seventeen conscripts gave blood every second month, the amount representing an average iron loss of 3.5 mg daily. Seven of them were given 20 mg and ten 100 mg iron as ferrous carbonate once daily throughout the study. Before the first and after the fourth and sixth blood donations they were examined with regard to packed red cell volume, serum iron, total iron-binding capacity and stainable bone marrow iron and diagnostic iron absorption test was performed. Ten conscript receiving no iron and giving no blood but comparable to those in the test groups with regard to age, exercise and diet served as controls. The stainable bone marrow iron was found to become stabilized at a level with reduced but still perceptible amounts when 20 mg iron was given and at a level with somewhat greater amounts when the daily dose was 100 mg. Both levels were lower than before blood donation in most subjects, and the negative iron balance was also reflected in the diagnostic iron absorption test. When healthy persons loses 3.5 mg iron daily supplementation with a 100 mg tablet per day is therefore insufficient to maintain the iron stores at their previous level. The decrease in storage iron is not progressive, however. When the storage iron is reduced iron absorption seems to be stimulated sufficiently to establish balance at reduced storage iron level.

Blood donors have been shown to have reduced iron stores (8, 13, 15, 24, 28, 33). Many investigators therefore recommend oral iron supplementation to prevent iron deficiency in blood donors (10, 17, 23, 26, 30, 37). The efficacy of this form of prophylaxis has been questioned. When as much as 2 000 mg iron was given within two weeks of each blood donation and when the interval between donations was two months the iron stores were lower in donors than in controls after only four blood donations (25). As each donation involves an iron loss of about 700 mg, less

than 10% of the supplied dose seems to have been absorbed.

Since the percentage absorption of iron from the intestine decreases with increasing doses (2,5) it seemed possible that a better result might be obtained if the iron were given in smaller doses throughout the entire period between donations.

The dose was calculated as follows. Six blood donations yearly (which is common in Swedish male donors) cause a total iron loss of about 1 700 mg, or an average loss of 3.5 mg daily. To compensate for this and the physiological daily loss of 0.6 mg (9) about 4 mg/day must be absorbed by male donors. Assuming the mean absorption to be 11-14% (5,27) the supplementation of the average food-iron intake of 15 mg/day (3, 6, 34) by 70 mg medicinal iron per day should cover the increased loss. The purpose of this study was to test the efficacy of the dose thus calculated. For comparison a large dose—100 mg daily—was also tested.

MATERIAL

Thirty conscripts aged 18-25 years, serving at the same regiment were assigned at random to two donor groups (the 20 mg and the 100 mg group) and one control group. During the study three members of the 20 mg group were posted to other regiments but none withdrew for other reasons. All had been found fit on physical examination before conscription, and none gave a history of bleeding or had previously donated blood.

METHODS

The packed cell volume (PCV) was measured by the routine technique in duplicate samples of capillary blood spun at 18 000 g for 6 min in microcapillary centrifuge.

The serum iron and total iron-binding capacity (TIBC) were determined by routine techniques in a Technicon AutoAnalyzer using the methods of Young and Hicks (15) as modified by Babson and Kleinman (1) for serum iron, and of Henry et al. (18) for TIBC. The samples were taken in the morning, the subjects having lain down for 5–10 min and having fasted overnight.

Stainable bone marrow iron was semiquantitatively assessed in bone marrow squash preparations made as described by Heesen and Weinfeld (12) and stained by the method of Rath and Finch (31). One examiner made estimations of 15–20 bone marrow fragments on each of two slides from each sternal puncture all on the same day. The slides were marked with code numbers, making it impossible for the examiner to identify them. The histochemical grades were defined as follows (11, 29, 31): 0=no stainable iron in any of the fragments, 1=small stainable granules not visible in every field ($\times 300$), 2=small stainable granules visible in every field, 3=numerous small granules, 4=numerous large granules, 5=numerous large granules with a tendency to form aggregates, 6=heavy deposits with aggregates.

The iron absorption test was done with radioactive iron and a whole-body counter. With the subjects fasting a dose of 0.25 mg FeSO_4 tagged with 0.5 μCi ^{59}Fe was given by mouth. The proportion of radioactivity retained in the body two weeks later is given as the result. The method, equivalent to what Heinrich (16) calls a "diagnostic" test designed to determine the degree of iron deficiency in a person, has been described in detail by Höglund (19). The test is not intended to measure the absorption from the large doses of medicinal iron given in this study.

Faecal iron was tested as described by Bonnar et al. (4). Primary tests on faeces from normal subjects taking carbonate pellets showed a strong positive reaction when the daily intake was 100 mg and a weak but still recognizable colour on an intake of 20 mg daily.

Design of the study

The PCV, serum iron, TIBC and stainable bone marrow iron were measured, and the diagnostic iron absorption test was done on all subjects at the beginning of the study. The donors then gave blood six times at mean intervals of 7.5 weeks. Reestimations of the iron variables were done after the fourth and sixth blood donations. The controls gave no blood and received no iron therapy; they were examined at the corresponding times. Radioiron for the iron absorption test was administered and samples for PCV, serum iron and TIBC determinations were taken on the same day. Sternal marrow samples were obtained on other occasions. The interval between the latest blood donation and the taking of blood samples varied and will be accounted for under Results.

Iron therapy The donor groups were given iron by mouth as ferrous carbonate stabilized with sugar in pellet form. The absorption from this particular preparation which prevents oxidation of the iron is reported to be equivalent to that from ferrous sulphate (21). The 20 mg group received one 20 mg tablet daily and the 100 mg

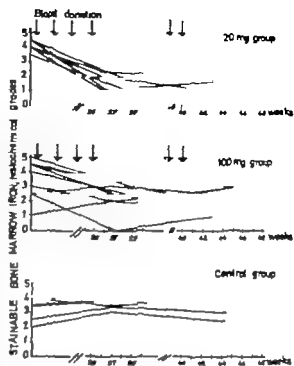


Fig. 1 Stainable bone marrow iron of different histochemical grades found initially and at various intervals after the fourth and sixth blood donations.

group one 100 mg tablet daily throughout the study except for five days before each iron absorption test. It was strongly and repeatedly emphasized to the participants that it was very important to take the tablets. Every participant was interviewed before each sternal puncture. One member of the 100 mg group had not tolerated the tablets well and had therefore not taken all of them. One-fourth of the others admitted to having forgotten no more than 8–10 tablets during the whole period. Faeces samples for qualitative iron analysis were brought by each subject two or three times during the study.

RESULTS

The findings initially and after four and six blood donations are shown in Fig. 1 and Table I. The intervals between the latest blood donation and blood sampling are shown in Table II.

Bone marrow examinations The amounts of stainable bone marrow iron are presented for each subject separately in Fig. 1. Initially the mean grade was 3.4 for the controls, 3.8 for the 20 mg group and 3.3 for the 100 mg group. Only 2 of 27 subjects showed a grade below 2.

Table 1 PCV serum iron TIBC and iron absorption in the three groups initially and after the fourth and sixth blood donations and differences in means values (the preinitial and last examinations compared in the initial values (d))

	Median time since last donation (d)	PCV (%)	Serum iron ($\mu\text{g}/100\text{ ml}$)	TIBC ($\mu\text{g}/100\text{ ml}$)	Diagnostic iron absorption test (%)
		Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
Controls (n=16)					
Initially		43.3 \pm 2.07	100 \pm 30	362 \pm 55	33.6 \pm 14.4
After 27 weeks		42.9 \pm 1.51	101 \pm 34	359 \pm 46	26.7 \pm 9.3
After 44 weeks		44.0 \pm 1.94	124 \pm 33	371 \pm 48	34.7 \pm 18.1
					- 6.9 NS + 1.1 NS
20 mg group (n=7)					
Initially		43.2 \pm 2.50	98 \pm 44	362 \pm 44	24.0 \pm 13.6
After 4 donations	43	42.3 \pm 3.34	89 \pm 34	377 \pm 50	46.3 \pm 21.2
After 6 donations	23	41.2 \pm 1.69	73 \pm 28	415 \pm 58	58.1 \pm 19.8
					+22.3 NS +34.1 NS
100 mg group (n=10)					
Initially		42.1 \pm 2.04	100 \pm 33	344 \pm 46	35.9 \pm 18.7
After 4 donations	21.5	41.3 \pm 1.86	111 \pm 48	367 \pm 53	52.1 \pm 22.5
After 6 donations	13.5	40.9 \pm 1.70	96 \pm 32	388 \pm 45	56.2 \pm 15.6
					+16.2 NS +20.3 NS

NS = not significant ($p > 0.05$) 0.05 < $p < 0.01$ 0.01 < $p < 0.001$ Student's *t*-test.

The control group showed essentially unchanged stainable bone marrow iron throughout the study. In no subject did the change exceed 1 histochemical grade. This suggests that the Swedish army diet provides iron in adequate quantity and form.

Both the 20 mg and the 100 mg groups showed a significant decrease from the initial gradings of 3.8 and 3.3 respectively to 1.6 and 2.4 respectively after the fourth blood donation. After the sixth donation the amount of stainable bone marrow iron was the same as after the fourth.

Iron absorption and variables in peripheral blood. In the donor groups, but not in the controls, the mean TIBC and the iron absorption as determined by the diagnostic test were found to be higher after both the fourth and sixth donations than initially. The increase is statistically significant for the findings after six donations and for TIBC also after four donations in the 20 mg group. The difference was greater after six donations than after four. The increases in TIBC and iron absorption were numerically greater in the 20 mg group than in the 100 mg group but the difference is statistically insignificant.

Faecal iron. Samples of faeces from each donor were examined for iron on two occasions during the study together with specimens from three control subjects to serve as negative faeces controls. In the 100 mg group 19 of 20 samples gave a positive reaction for iron. In the 20 mg group 9 of 14 specimens were positive. The five negative samples were produced within one week of a blood donation, but subsequent samples from the same subjects showed positive reactions for iron.

DISCUSSION

The high blood donation rate in this study would have practically deprived the subjects of stainable bone marrow iron after only four donations if additional iron had not been given (25). Nevertheless the mean stainable bone marrow iron decreased. Furthermore the diagnostic iron absorption test and the TIBC showed changes usually ascribed to iron deficiency. Several explanations are conceivable for the latent iron deficiency that persisted despite prophylactic iron therapy.

1 The iron deficiency may be only apparent, and the signs of iron deficiency may be a result of too short intervals between donations and laboratory tests. The greater increase in TIBC and

Table II Interval between latest blood donation and laboratory tests

	After four donations (d.)		After six donations (d.)	
	20 mg group	100 mg group	20 mg group	100 mg group
Sternal puncture				
Range	8-22	8-28	19-48	16-48
Mean	12.3	15.3	32.9	27.2
Median	9	16	39	20
Absorption test and peripheral blood values				
Range	11-44	7-43	7-34	4-39
Mean	36.4	24.3	20.4	18.6
Median	43	22	24	13

iron absorption in both donor groups after six donations than after four can for example be explained by the shorter interval after the sixth donation. However, the very fact that the signs of iron deficiency persist for nearly one year when the mean interval between donation and test was as long as 36.4 days (Table II) suggests that latent iron deficiency is actually present most of the time.

2. Donors may have neglected to take their iron. The interviews with the donors and the faecal suggest a certain neglect. Even if it is assumed that 15% of the tablets had in fact not been taken the 20 mg group was nevertheless receiving an average of 17 mg iron per day and the 100 mg group an average of 83 mg per day.

3. There may be unsatisfactory absorption of the iron from the tablets taken. Even with the above reservations in mind it seems difficult to reach any other explanation. Probably less than 70% and 4% of the 70 mg and 100 mg tablets respectively was absorbed. The proportion may appear low but tallies with data given by Heinrich (16) from which it can be inferred that only 4-5 mg can be expected to be absorbed from a single 100 mg dose in men with "normal" iron stores. In our investigation repeated doses were given and still less of the iron could therefore be expected to be absorbed owing to the mucosal block (7, 20).

The latent iron deficiency found does not appear to be of a progressive nature but a balance seems to be established. The fact that the iron state after six blood donations was the same as after four

suggests that the iron lost at the fourth and fifth donations was regained before the next one in both groups. The total quantity of iron absorbed from food and tablets must therefore have been about 4 mg/day both in the subjects taking 20 mg and in those taking 100 mg iron daily.

The 20 mg group thus balances at a level characterized by low storage iron and a pathological diagnostic iron absorption test; the 100 mg group balances at a higher level with less abnormal values. This can be explained by the inverse relationship between iron absorption and the size of the iron stores shown by Hausmann et al. (14) and Höglund et al. (22). When the stores are low a greater proportion of the iron dose is absorbed, and at a certain point balance is achieved.

In conclusion, 20 mg ferrous iron daily to blood donors is enough to compensate for the iron lost by blood donation only if reduced bone marrow iron stores are accepted. If larger stores are aimed at, much greater doses must be given: even a dose of 100 mg daily (36 000 mg yearly) seems to be too little to maintain iron stores at their predonation levels. It is doubtful whether such huge doses would be realistic in blood bank practice.

ACKNOWLEDGEMENT

This study was supported by a grant from AB Astra Södertälje.

REFERENCES

- Babson, A. L. & Kleinman, H. M. A source of error in an AutoAnalyzer determination of serum iron. *Ch. Chem.* 13, 163, 1967.
- Bennerman, R. M. Iron absorption and excretion in experimental iron deficiency. *Nucl. Med. Suppl.* 1, 77, 1961-6.
- Blax, O., Järret, K. & Löfdgren, L. *Ärskost och järnterapi* pp. 66-72. Lindgren & Söner Göteborg, 1963.
- Bosmar, J., Goldberg, A. & Smith, J. A. Do pregnant women take their iron? *Lancet* i, 457, 1969.
- Bothwell, T. H., Pizzo-Biroh, G. & Finch, C. A. Iron absorption. I. Factors influencing absorption. *J. Lab. clin. Med.* 51, 4, 1958.
- Carlgen, O. & Cramer, K. Förändringen i järnstatus i relation till totala kalorietaget. *Årskartidningen* 6, 4248, 1965.
- Crosby, W. H. Intestinal response to the body requirement for iron. *J. Amer. med. Ass.* 208, 347, 1969.

8. Fielding, J., Karabus C. & Brunström, B. M. Storage iron depletion in male blood donors: its significance for iron status in women. *J clin. Path.* 21 402, 1968.
9. Fisch, C. A. Body iron exchange in man. *J clin. Invest.* 38 392, 1959.
10. French, E. A. & Sittler, P. K. Plasma iron values in national blood transfusion service donors. *J clin. Path.* 22 680 1969.
11. Gale, E., Torrance, J. & Bothwell, T. The quantitative estimation of total iron stores in human bone marrow. *J clin. Invest.* 42: 1076, 1963.
12. Hansen, H. A. & Weinfeld, A. Hemosiderin excretions and sideroblast counts: the differential diagnosis of iron deficiency and other anemias. *Acta med. scand.* 165 333 1959.
13. Hawkins, D., Stevens, A. R., Fisch, S. & Fisch, C. A. Iron metabolism. Iron stores in man as measured by phlebotomy. *J clin. Invest.* 31 543 1952.
14. Hasselmann K., Kuse R., Sosenberg, O. W., Barich, H. & Helrich H. C. Inter-relationships between iron stores, general factors and intestinal iron absorption. *Acta haemat. (Basel)* 42, 193 1969.
15. Heinrich, H. C. Prälatente latente und manifeste Eisenspeicherzustände bei Blutspendern. *Munch. med. Wochschr* 110: 1845 1968.
16. — Intestinal iron absorption in man. In: Iron deficiency (ed. L. Hallberg, H.-G. Harwerth & A. Vannotti) pp 213-234. Academic Press, London and New York 1970.
17. Heinrich H. C., Oppatz, K.-H. & Busch, H. Eisenspeicher und Eisenprophylax bei Blutspendern. *Klin. Wochschr* 51 101 1973.
18. Henry R. J., Sobel, Ch. & Chasmon, N. On the determination of serum iron and iron-binding capacity. *Clin. chim. Acta* 3 523 1958.
19. Höglund S. Iron absorption in apparently healthy men and women. *Acta med. scand.* 186, 487 1969.
20. — Studies in iron absorption. VI Transitory effect of oral administration of iron on iron absorption. *Blood* 34 505 1969.
21. Höglund S., Elm, L. & Enander H. Resorptionen av järn från ferrokarbonat. *Opusc. med.* 18 12, 1973.
22. Höglund S., Elm, L. & Ljeden, G. Studies in iron absorption. VII Iron deficiency in young men. *Acta haematol.* 44 193 1970.
23. Kwa, S. B. The effect of repeated blood donation on the haemoglobin, haematocrit and serum iron values of regular blood donors. *Singapore med. J.* 10: 139 1969.
24. Ljeden, G. Iron state in regular blood donors. *Scand J Haemat.* 11 342, 1973.
25. — Iron supplement to blood donors. I. Trials with intermittent iron supply. *Acta med. scand.* 197 31 1975.
26. Marthes, M. & Kramer H. Der Eisenbedarf des Blutspenders. *Dtsch. med. Wochschr* 81 1262, 1956.
27. Moore, C. V. Iron nutrition. In: Iron metabolism (ed. F. Gross), pp. 241-255. Springer Verlag, Berlin Göttingen and Heidelberg 1964.
28. Möller H. Untersuchungen am Blutspender insbesondere Serum Eisen- und Serumprotein-Bestimmungen. *Z. ges. inn. Med.* 7 341 1952.
29. Pritchard, J. A. & Scott, D. E. Iron demands during pregnancy. In: Iron deficiency (ed. L. Hallberg, H.-G. Harwerth & A. Vannotti) pp 173-180. Academic Press, London and New York 1970.
30. Ramgren, O. & Tengberg, J.-E. Profylaktisk järntillförsel till blodgivare. *Nord. Med.* 65 488, 1961.
31. Rath, C. & Fisch, C. A. Sternal marrow hemosiderin. *J. Lab. clin. Med.* 33 11 1948.
32. Schneider W., Böwing, H. & Teixeira, J. Ergebnisse der Eisenprophylax bei Blutspendern. *Medizinische* 1 782, 1959.
33. Weinfeld, A. Storage iron in man. *Acta med. scand., Suppl.* 427 1965.
34. Wretling, A. Food iron supply. In: Iron deficiency (ed. L. Hallberg, H.-G. Harwerth & A. Vannotti), pp 39-64. Academic Press, London and New York 1970.
35. Young, D. S. & Hicks, J. M. Method for the automatic determination of serum iron. *J. clin. Path.* 18 98 1965.

DEATHS FROM ISCHAEMIC HEART DISEASE IN HELSINKI IN THE YEARS 1959-1968

Vital Statistics and Medico-legally Autopsied Sudden Deaths

Viljo Rissanen Martti Tenhu and Unto Uotila

*From the Second Department of Medicine and the Department of Forensic Medicine
University of Helsinki, Helsinki, Finland*

Abstract According to the official vital statistics, altogether 10910 deaths from ischaemic heart disease (IHD) occurred in persons resident in Helsinki during the 10-year period 1959-68. A significant increase was found in the incidence of IHD deaths in both sexes even though changes in the structure of the population were taken into account. The increase in the age-dependent incidence of IHD deaths was most conspicuous at middle age in both sexes and in young males. Altogether 3044 IHD deaths occurring unwitnessed or within 24 hours of the onset of the fatal attack were autopsied medico-legally during the 10-year period of the study. The medico-legally autopsied cases obviously represented a high proportion of sudden IHD deaths occurring outside hospitals in Helsinki. A clear male preponderance was found in the autopsy material as compared with all IHD deaths. The prevalence of cases of acute myocardial infarction varied in different years from 27 to 49% at medico-legal autopsies. No significant change occurred during the 10-year period in the distribution of the medico-legally autopsied IHD deaths into social groups, in the residences or place of death. The home was the most common place of death. From 1963 onwards the patients dying from an ischaemic heart attack during transportation to hospital or in an outpatient department constituted 6-7% of all annual IHD deaths.

According to the clinical epidemiological prospective studies (10-12) and vital statistics (35) Finland is one of the leading countries in both morbidity and mortality from ischaemic heart disease (IHD). Studies from previous decades suggest that IHD mortality has increased continuously in Finland (6, 7, 30-32). Nowadays the population of Helsinki, the capital of the country, certainly

represents a mixed Finnish population owing to brisk migration. Thus the disease situation in Helsinki may present a good cross-section of the situation in the country.

Suddenness of death is, without doubt, one of the most difficult problems associated with IHD. The essential role played by IHD as a cause of sudden death is evident in many postmortem series varying from 50 to 91% of sudden natural deaths (14, 15, 28, 31). A great majority of sudden IHD deaths occur outside hospitals and before any medical care is available. This was also shown in an extensive epidemiological cross-sectional and retrospective study performed in Helsinki in 1970 by the Ischaemic Heart Disease Register (25, 26). The study showed that about 80% of IHD deaths in Helsinki are confirmed at autopsy. The most problematic sudden IHD deaths in Finland, those occurring outside hospitals, are autopsied medico-legally. In Helsinki all medico-legal autopsies are performed in the Department of Forensic Medicine, University of Helsinki. Since there has been no organized register system in Helsinki for the collection of the data related to IHD deaths before the Ischaemic Heart Disease Register was established and started in 1969, it is obvious that the most suitable way to obtain information on the sudden IHD deaths which have occurred during previous years is to analyze retrospectively the medico-legally autopsied cases. Since the autopsy rate is so high, one can expect that these patients form a rather representative sample of sudden IHD deaths occurring outside hospitals in Helsinki.

The present study was performed in order to

Requests for reprints to: V. Rissanen, Second Department of Medicine, University Central Hospital, SF-00290 Helsinki 29, Finland.

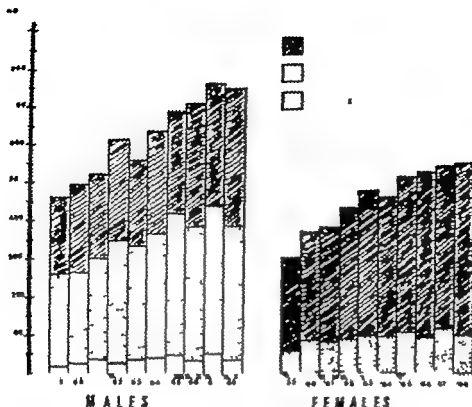


Fig. 1 Deaths from IHD by age and sex in Helsinki during 1959-68

to trace the history of IHD mortality in Helsinki during the 10-year period 1959-68. In addition, a more detailed analysis of the medico-legally autopsied sudden IHD deaths from the same period is presented. The present paper deals with the official vital statistics and certain factors related to the history of the fatal attack and social background of the medico-legally autopsied sudden IHD deaths.

MATERIAL AND METHODS

Vital statistics

The results concerning IHD mortality in Helsinki are based on the official vital statistics obtained from the Central Statistical Office of Finland. The principal source of this information is the death certificates gathered at the Statistical Office annually from the whole country. During the 10-year period of the study the nomenclature of the diseases was based in Finland on the International Classification of Diseases from 1952. The cases in which the underlying cause of death was coded 430, IHD, were taken as the object of the study.

Medico-legal material

The medico-legal material consist of subjects autopsied in the Department of Forensic Medicine, University of

Helsinki, during 1959-68 for whom the cause of death was IHD and the death had not been witnessed or the fatal attack had terminated within 24 hours of the onset of symptoms. Only those who resided in Helsinki at the time of death were included.

According to WHO rules the diagnosis of IHD death is definite at autopsy if a recent myocardial infarct and/or a fresh coronary thrombosis can be demonstrated (34). The prevalence of medico-legally autopsied cases fulfilling these criteria of acute myocardial infarction (AMI) was analysed annually on the basis of the autopsy records. The diagnosis of a recent infarct was based mainly on macroscopic findings, but also in certain cases on conventional histology or on macroscopic histochemical staining of myocardial specimens (19). The histochemical method was applied to routine autopsy work in the Department during 1966-68.

The division of the medico-legally autopsied subjects into three social groups was based on the subject's occupation. The criteria presented by Raohala (22) were followed the principles being in common use in Finland.

The history of the fatal attack was gained from the police report in all cases and from the hospital records if the patient had been transported to a hospital or to an outpatient department.

The autopsied subject were divided into three groups according to the suddenness of the death: 1) deaths occurring within 1 hour of the onset of symptoms, 2) deaths occurring within 1-4 hours of the onset of

Table I Total mortality rate in relation to deaths from IHD per 1000 inhabitants per year during 1959-68 in Helsinki

	Males			Females		
	Total	IHD	IHD/total (%)	Total	IHD	IHD/total (%)
1959	9.95	2.39	24.0	8.58	1.24	14.5
1960	10.39	2.52	24.3	8.96	1.50	16.7
1961	10.38	2.61	25.2	9.17	1.53	16.7
1962	10.42	2.98	28.7	9.78	1.70	17.4
1963	10.13	2.64	26.0	9.49	1.89	19.8
1964	10.23	2.95	28.9	9.24	1.73	18.7
1965	10.78	3.11	28.9	9.39	1.90	20.2
1966	10.32	3.12	30.3	9.30	1.89	20.3
1967	10.79	3.31	30.8	9.04	1.92	21.2
1968	10.52	3.21	30.5	8.98	1.93	21.5

symptoms, 3) unwitnessed deaths in which the duration of the terminal attack was not exactly known. Probably most deaths in the third group were also sudden, occurring at least within 24 hours of the onset of symptoms.

The place of death was grouped as: 1) home, 2) transit or an outpatient department, 3) other places (at work, in the street, etc.) including hospital departments (86 persons, 2.8% of the entire material).

RESULTS

Vital statistics

During the 10-year period 1959-68 altogether 10910 fatal attacks due to IHD were recorded among residents of Helsinki. The absolute number of IHD deaths increased during this period in all age groups (Fig. 1). The average population of Helsinki increased considerably and equally in both sexes during the same period from 442800 in 1959 to 529200 in 1968. The total mortality and the mortality from IHD per 1000 inhabitants per year are given in Table I. Only a minimal increase if any was observed during the 10-year period in the incidence of all deaths. On the other hand the incidence of IHD deaths increased

34.3% in males and 55.7% in females. The proportion of IHD deaths in relation to all deaths increased from 24.0% in 1959 to 30.5% in 1968 in males and from 14.5% in 1959 to 21.5% in 1968 in females. The age-dependent incidences of IHD deaths from 1961 and 1967 are given in Table II. The increase in the age-dependent IHD incidence was most conspicuous in middle-aged males and females and in young males being about similar in females aged 45-64 and in males aged 25-44 and 45-64 years.

Medico-legal material

During the 10-year period 1959-68 altogether 3044 fatalities among residents of Helsinki, occurring unwitnessed or within 24 hours of the onset of the fatal attack, i.e. 28% of all IHD deaths were diagnosed as IHD deaths at autopsies performed in the Department of Forensic Medicine, University of Helsinki. The medico-legally autopsied cases fulfilling the criteria of the study formed an increasing proportion of all IHD deaths: 19.6% in 1959 and 33.6% in 1968 (Table III). The number of medico-legally autopsied males during the 10-year period was 2009 and that of

Table II. Age-dependent incidence of IHD deaths per 1000 inhabitants per year in Helsinki in 1961 and 1967

Age (y)	Males			Females			Total		
	1961	1967	Increase (%)	1961	1967	Increase (%)	1961	1967	Increase (%)
25-44	0.46	0.67	45.7	0.10	0.09	-	0.26	0.36	38.5
45-64	5.81	8.23	41.6	1.12	1.64	45.3	2.83	4.28	51.3
≥65	21.9	24.8	13.2	11.2	13.0	16.1	14.0	16.4	17.1

Table III. Medico-legally autopsied sudden IHD deaths during 1959-68 in Helsinki: the proportion of these cases in relation to all IHD deaths, the prevalence of AMI cases, the distribution of cases into social groups, suddenness and place of death, and the deaths in transit or in outpatient departments as a percentage of all IHD deaths (2/7)

	No. of cases	Proportion of all IHD deaths	Prevalence of AMI cases	Social class			Suddenness of death (group)			Place of death (group)			
				I	II	III	1	2	3	1	2	3	2/7
1959	152	19.8	77.0	13	36	51	64	22	14	61	20	19	4.0
1960	198	22.6	76.4	18	30	52	59	29	12	57	22	21	4.9
1961	700	21.6	42.0	13	35	52	54	23	23	64	16	20	3.4
1962	276	25.9	45.3	12	33	55	74	11	15	69	14	17	3.6
1963	264	25.0	32.6	14	31	55	62	15	23	53	27	20	6.6
1964	321	28.8	35.5	14	33	53	58	19	23	60	23	17	6.5
1965	365	29.8	35.4	15	34	51	55	18	27	62	23	15	8.8
1966	384	30.6	49.0	13	32	55	58	16	26	57	23	20	6.8
1967	438	33.1	46.0	12	34	54	63	16	21	62	20	18	6.7
1968	445	33.6	48.8	16	37	52	52	34	14	64	23	13	7.7
Total	3044	27.9	41.3	14	33	53	60	19	21	61	1	19	5.9

Division into groups, see Material and methods.

females 1035. The male:female ratio was thus 1.9:1 (Table IV). According to official vital statistics the corresponding sex ratio for all IHD deaths in the 10-year period was 1.3:1. Males were overrepresented in the medico-legal material in all age groups (Table IV). The absolute number of IHD deaths was greater in males than

females below the age of 65 years, while a sex ratio was found at older ages both for IHD deaths and for medico-legally autopsied cases (Table IV). The age-dependent incidence of all IHD deaths per 1000 inhabitants of the same age in the Helsinki population was however clearly higher in males than in females in all age groups (Table II). About 53% of all IHD deaths among subjects below 45 years had been autopsied medico-legally, while the corresponding autopsy rate was 36% in the age group 45-64 and 21% in the age group 65 and above.

Table IV. Sex ratio for all IHD deaths and for the medico-legal autopsy material by age, the years 1959-68 combined

Age (yr)	Total (8/9)	Medico-legal autopsy material (8/9)
<44	6.1/1	6.7/1
45-64	3.3/1	3.8/1
≥65	0.7/1	0.9/1
Total	1.3/1	1.9/1

The prevalence of AMI cases according to WHO criteria varied in different years from 27 to 49% at medico-legal autopsies (Table III). During the last three years when the macroscopic histochemical staining of the myocardium had become a routine autopsy method in this Department the prevalence of detected recent infarcts was highest.

No significant change had occurred during the period in the distribution of medico-legally autopsied IHD deaths into the three social groups (Table III). The distribution of males into the social groups was clearly different from that of females (Table V). The estimation of the social group was probably erroneous in many females due to the high prevalence of unspecific occupations such as housewife etc. No consistent difference was found in males in the distribution into social groups in different age groups. The small number of females in the younger age groups makes the interpretation of the significance of the age group differences in them difficult.

The distribution of the medico-legally autopsied cases into the analysed groups of suddenness of death did not change during the period (Table III). In about 80% of these IHD deaths the fatal attack had been unwitnessed or had terminated within 1 hour of the onset of symptoms (Table VI). In both males and females unwitnessed deaths were more common after the age of 65 than at younger ages. The death had been unwitnessed more often in females than in males.

Table V Distribution of medico-legally autopsied sudden IHD deaths into three social groups (I-III) by age and sex the years 1959-68 combined (data for social group available in 2970 cases)

Age (y.)	Males							Females						
	I		II		III		Total	I		II		III		Total
	N	%	N	%	N	%		N	%	N	%	N	%	
<44	188	40	21	73	39	75	40	28	1	3	8	29	19	68
45-54	448	78	17	188	42	182	41	82	3	4	33	28	56	68
55-64	697	110	16	273	39	314	45	226	11	5	50	22	165	73
≥65	629	126	20	247	39	256	41	672	41	6	125	13	506	75
Total	1962	354	18	781	40	827	42	1008	56	6	206	20	746	74

The witnessed attacks terminating within 1 hour were slightly more common in males. About 20% of both sexes had survived more than 1 hour after the onset of symptoms.

No significant tendency to a change in the distribution of medico-legally autopsied cases into the groups of place of death was found during the 10-year period (Table III). It is noteworthy that the proportion of patients dying during transportation to a hospital or in outpatient departments was rather constant in relation to the number of all IHD deaths. From 1963 these cases constituted 6.5-7.7% of all annual IHD deaths. In absolute figures this signified an increasing number of patients per year from 70 in 1963 to 102 in 1968. The frequency of deaths occurring in transit or in outpatient departments was not dependent on age, but was higher in males than in females (Table VII). The home was the most usual place of death, more often in females than in males and more often in elderly than in younger males.

DISCUSSION

The incidence of deaths from IHD and the proportion of these deaths in relation to all causes of death have increased considerably among the population of Helsinki during the 10-year period of the study. The increase is valid even though the changes in the number and composition of the population are taken into account. It is noteworthy that the increase in age-dependent IHD mortality has been highest among persons of middle age. The increasing tendency to IHD mortality has also been found in several other countries (2, 3, 11, 18). On the other hand some authors have criticized the results based on vital statistics and have suggested that there is no evidence of an increasing incidence of IHD, e.g. in England and the USA, and that the changes in the age structure of a population and in diagnostic habits may largely explain the increasing prevalences of the disease (24). From previous studies it is well known that mortality from IHD is highest in the eastern parts of Finland (10, 22). It was

Table VI Prevalence (%) of subjects dying unwitnessed within 1 hour and 1-24 hours of the onset of the fatal attack in the medico-legal autopsy material during 1959-68 by age and sex

Age (y.)	Males (group)						Females (group)*					
	1		2		3		1		2		3	
	Total	N	%	N	%	N	Total	N	%	N	%	N
<44	188	126	67	40	21	22	33	21	75	4	18	3
45-54	490	297	66	83	19	68	82	44	54	21	25	17
55-64	717	473	66	136	19	108	228	138	56	40	21	52
≥65	654	406	62	118	18	130	697	327	47	140	20	230
Total	2009	1302	65	379	19	328	1035	520	50	213	21	302

* Division into groups, see Materials and methods.

Table I Age and sex distribution of persons autopsied in pathologic or forensic departments and of non-autopsied deaths

Age (y)	Recent changes at autopsy ^a								Clinical data			
	Pathologic departments				Forensic departments				Non-autopsied deaths			
	Total	Definite	Probable	None	Total	Definite	Probable	None	Total	Definite	Possible	Insufficient data
Males												
-44	11	7	2	2	29	7	10	12	1	-	1	-
45-54	28	20	3	5	54	19	15	20	10	-	3	5
55-65	92	58	18	16	121	29	45	47	62	11	12	39
Total	131	85	23	23	205	56	70	79	73	13	16	44
Females												
-44	1	1	-	-	3	-	2	1	-	-	-	-
45-54	4	4	-	-	12	2	5	5	1	1	-	-
55-65	35	23	7	5	41	11	15	15	20	9	4	7
Total	40	28	7	5	56	13	22	21	21	10	4	7
Both sexes	171	113	30	28	261	69	92	100	94	23	20	51

^aAn acute infarction or a fresh coronary thrombus.

deaths autopsied in the pathologic departments of hospitals or in the Department of Pathology, University of Helsinki, were dealt with separately from those autopsied medico-legally. About half of all fatal cases had been autopsied medico-legally and one third in the pathologic departments. The remaining 18% of IHD deaths were recognized without autopsy.

The reliability of the diagnosis of IHD death (Table I) determined by the instructions laid down by WHO (1). The categories were based in autopsied cases on postmortem evidence of an acute ischaemic attack. A "definite" death from IHD was a case in which either a macroscopic necrotic infarction was found at autopsy or a fresh coronary artery thrombosis was demonstrated. The "probable" category included cases in which a non-necrotic macroscopic appearance of a recent infarction was observed with the naked eye and/or infarct changes were demonstrated by histology. The "none" category in autopsied cases comprised those in which no evidence of an acute infarction or thrombosis could be obtained at autopsy. In non-autopsied cases the diagnostic categories were based on clinical data (22). In "definite" case changes in ECG or serum enzymes were reported, the diagnosis possibly being supported by a history of typical chest pain. The "possible" infarctions were those with a typical history of chest pain in association with the attack, while ECG or serum enzyme evidence was lacking. "Insufficient data" were those in which none of the above mentioned clinical evidence was obtained, but no diagnosis other than that of IHD was made. Definite or probable patho-anatomic evidence of an acute heart attack was obtained in 84% of persons autopsied in the pathologic departments and in 83% of those autopsied medico-legally. A history of typical chest pain in association with the fatal attack or of a previous IHD was known in 25 of 28 persons in the "none" category in the pathologic department series

and in 70 of 100 persons in the "none" category in the medico-legal series. Definite and possible clinical categories were obtained in 46% of non-autopsied cases. A history of a previous IHD was known in 42 of 51 persons in the "insufficient data" category. Hence, the diagnosis of IHD death was either based on the positive patho-anatomic or clinical evidence of an acute heart attack or else supported by a history of clinical IHD in no less than 92% of all registered fatalities.

The majority of deaths autopsied in the pathologic departments were delayed while most persons autopsied medico-legally had died suddenly or unwitnessed (Table II). Of 171 persons autopsied in the pathologic departments 163 (95%) and of 94 non-autopsied persons 34 (36%) had died in a hospital. Of 261 medico-legally autopsied persons 242 (93%) had died outside hospitals.

Social groups

The division into four social groups was based on the person's occupation (22). The same division has been used in the census in Finland e.g. by the Statistical Office of the City of Helsinki (23).

Disease history

Information on previous diseases was obtained by the interviewers from the patients or their relatives and from hospital records. The history of a previous myocardial infarction is given in this paper according to two categories, "confirmed" and "non-confirmed" suggested by the WHO. The history of a previous angina pectoris was based on the definition of the WHO (31). The persons were in this respect divided into those who had suffered from angina for more than 28 days before death and those whose first anginal symptoms appeared within 28 days preceding death. The level of medical care was evaluated by recording the visits to a doctor within 28 days preceding death.

Table II Suddenness of autopsied and non-autopsied deaths

	Total no. of pats.	Less than 1 h		1-24 h		24 h-28 d.		Unwitnessed		Insuff. data
		No.	%	No.	%	No.	%	No.	%	
Pathologic departments										
Males	131	27	21	26	20	75	57	0	0	3
Females	40	3	7.5	13	32.5	22	55	1	2.5	1
Forensic departments										
Males	205	130	63	17	8	26	13	32	16	0
Females	56	30	54	9	16	7	13	10	18	0
All autopsied cases	432	190	44	65	15	130	30	43	10	4
Non-autopsied deaths										
Males	73	41	56	10	14	17	23	5	7	0
Females	21	6	29	3	14	10	48	2	10	0
Total	94	47	50	13	14	27	29	7	7	0

Visits due to premonitory symptoms were analysed separately. An attempt was made to clarify the previous medication of patients, particular attention being focused on the use of digitalis.

Autopsy data

All departments of pathology and forensic medicine in Helsinki were informed of the study. Detailed instructions were given by the Registrar team to the pathologists to complete a special record form whenever a fresh myocardial infarction was found or in cases when ischemic heart disease was considered to be an essential cause of death. Routine autopsy technique was employed in the Departments of Pathology, Astoria, Kivela and Maria Hospitals and in the Department of Pathology, University of Helsinki. Patients dying in Meilahti Hospital, the biggest hospital in the city, are autopsied in the Pathologic Department of the University. The results of histologic examinations at 29 autopsies in the pathologic departments were reported.

A number of 248 medico-legally autopsied persons had been examined in the Department of Forensic Medicine, University of Helsinki. A member of the study team carried out the examination of the hearts in 194 registered IHD deaths. A special methodology suggested by the WHO scientific group (32) was applied to the investigation of these cases, which constituted 74% of the medico-legal series. A double contrast modification of postmortem coronary angiography was developed and used for the demonstration of changes in the coronary arteries (20). In addition, the longitudinally opened coronary arteries were carefully examined. The heart was sectioned into slices from the apex to the base. The slices were stained by a macroscopic histochemical method (19) for demonstration of infarcted areas. Myocardial blocks were taken for histology from macroscopically suspected areas or if the macroscopic examination was negative from the anterior and posterior wall of the left ventricle and from the interventricular septum. The principles adopted in the WHO Cooperative Study (33) were followed when making the histologic diagnosis of recent infarct lesions. In 67 medico-legally autopsies the patho-anatomic findings of the

heart were based on routine autopsies, the record forms being completed by a member of the team.

A recent myocardial infarction was regarded as definite when macroscopic necrosis was observed with the naked eye in the myocardium (34). The ruptured infarctions were recorded separately. The infarction was "probable" when discoloration, pallor or softening but no necrosis was observed in a demarcated area of the myocardium and/or histologic changes of an acute infarction were demonstrated by microscopic examination. The location of an infarction was indicated by drawings (Fig. 1). A scar 0.5 cm or more in diameter was regarded as an old healed infarction (8). The location of scars was also shown by drawings (Fig. 2). A slight fibrosis less than 0.5 cm in diameter was recorded separately. The heart had been weighed at autopsy in 403 cases. The presence of fresh coronary thrombus was estimated visually. The degree of coronary stenosis was also reported in record forms. Since the estimations made by several pathologists are not truly comparable, only the reported frequencies of coronary occlusions are given.

The statistical computations of this study were performed with Burroughs 6700 computer in the Computing Centre of the University of Helsinki. The statistical methods and tests used in this study are standard and commonly known.

RESULTS

Social groups

All registered cases of acute ischemic heart disease (AIHD) and all autopsied cases showed a division into four socio-economic groups very similar to that of the population of Helsinki according to the census made in 1965 (Table III). Men belonging to the highest social group (I) were under-represented and those of the lowest social group (IV) over-represented among medico-legally autopsied cases.

Posterior



Anterior

PATHOLOGIC DEPARTMENTS

Posterior



Anterior

Posterior



Anterior

FORENSIC DEPARTMENTS

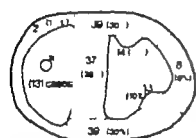
Posterior



Anterior

Fig 1 Prevalence of recent infarct changes in various segments of the wall of cardiac chambers in males and females autopsied in the pathologic and forensic departments.

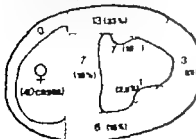
Posterior



Anterior

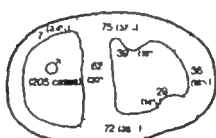
PATHOLOGIC DEPARTMENTS

Posterior



Anterior

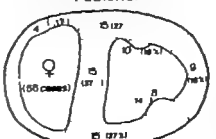
Posterior



Anterior

FORENSIC DEPARTMENTS

Posterior



Anterior

Fig 2 Prevalence of old infarct scar in various segments of the wall of cardiac chambers in males and females autopsied in the pathologic and forensic departments.

Table III. Distribution of autopsied and non-autopsied deaths and all registered cases of AIHD into four socio-economic groups and the corresponding distribution of males and females aged 15 years and above in the population of Helsinki

	Total no of pats.	Group I		Group II		Group III		Group IV		Unknown	
		No.	%	No.	%	No.	%	No.	%	No.	%
Pathologic departments											
Males	131	27	21	24	18	58	44	22	17	11	
Females	40	1	2.5	11	25	19	47.5	10	25	0	
Forensic departments											
Males	205	26	13	47	23	87	43	41	20	4	2
Females	56	5	9	9	16	24	43	15	28	3	5
All autopsied deaths	432	59	14	90	21	188	44	88	1	7	2
Non-autopsied deaths											
Males	73	19	26	21	29	74	33	6	8	3	4
Females	21	3	14	2	10	10	48	6	29	0	
Total	94	22	23	23	25	34	36	12	13	3	3
Registered cases of AIHD											
Males	953	187	20	197	21	41	44	149	16	9	1
Females	314	25	8	62	20	143	46	78	25	6	2
Population of Helsinki											
Males			19		21		43		16		0.3
Females			7		19		49		25		0.3

while among non-autopsied persons the situation was the opposite. A relatively greater proportion of acute attacks of IHD in persons belonging to social group IV had terminated fatally (44%) than in persons of social group I (38%). The difference was not, however, significant. In the social groups II and

III the corresponding frequencies were 44% in the former and 40% in the latter.

Disease history

A previous infarction was known more often in men autopsied in the pathologic departments than in men

Table IV. Prevalence of the history of a previous myocardial infarction, angina pectoris more than 28 days before death and first anginal symptoms within 28 days preceding death in autopsied and non-autopsied deaths and all registered cases of AIHD

	Total no. of pats.	Previous infarction			Angina > 28 d.			Angina < 28 d.		
		Data available	No. of cases	%	Data available	No. of cases	%	Data available	No. of cases	%
Pathologic departments										
Males	131	129	76	59	127	94	74	127	7	6
Females	40	40	15	38	39	30	77	36	1	3
Forensic departments										
Males	205	162	50	31	151	94	62	149	4	3
Females	56	43	16	37	40	27	67	36	1	3
All autopsied deaths	432	374	157	42	357	245	69	348	13	4
Non-autopsied deaths										
Males	73	61	29	47	65	54	83	58	2	3
Females	21	18	3	17	17	9	53	14	1	7
Total	94	80	32	40	82	63	77	72	3	4
All registered cases of AIHD	1 267	1 177	425	40	1 162	802	69	1 145	111	11

Table V Visits to a doctor and visits due to premonitory symptoms within 28 days preceding death and the use of digitalis in autopsied and non-autopsied deaths and all registered cases of AIHD

	Visits to a doctor				Use of digitalis			
	Data available	All visits		Premonit. sympt.		Data available	No. of cases	
		No. of cases	%	No. of cases	%		No. of cases	%
Pathologic departments								
Males	125	81	65	34	27	125	75	60
Females	37	29	78	12	32	40	26	65
Forensic departments								
Males	143	65	45	23	16	157	40	25
Females	40	19	48	7	17	43	20	47
All autopsied deaths	345	194	56	76	22	365	161	44
Non-autopsied deaths								
Males	62	39	63	15	24	63	28	44
Females	17	6	35	1	6	16	11	69
Total	79	45	57	16	20	79	39	49
All registered cases of AIHD	1 136	481	42	204	18	1 127	469	42

autopsied medico-legally (Table IV). No corresponding difference was found in women in whom the overall prevalence of a previous infarction was lower than in men. Angina pectoris more than 28 days before death was even slightly more common in men than in women among those autopsied but autopsied cases showed an opposite trend. A history of a previous angina was more common in autopsied in the pathologic departments than in those autopsied medico-legally ($p < 0.5$). Of non-autopsied fatal cases 80% had a history of symptomatic heart disease before death. A new angina pectoris appearing within 28 days preceding death was rather uncommon in all groups. It was

slightly less common among fatal cases of AIHD than among all persons suffering from an acute attack of IHD during the study period.

Persons who died had visited a doctor more often within 28 days preceding death and visits due to premonitory symptoms were slightly more common than among all registered cases of AIHD (Table V). All visits ($p < 0.01$) and visits due to premonitory symptoms ($p < 0.1$) were more common in persons autopsied in the pathologic departments than in those autopsied medico-legally. The use of digitalis was clearly rarest in men autopsied medico-legally and most common in non-autopsied women and persons autopsied in the pathologic departments.

Table VI Prevalence of a recent myocardial infarct in persons autopsied in the pathologic and forensic departments

	Necrosis		Rupture		Probable		No infarct		Insuff. data	
	No.	%	No.	%	No.	%	No.	%	No.	%
Pathologic departments										
Males	62	47	10	8	33	23	19	15	7	5
Females	23	58	4	10	7	17	6	15	0	
Forensic departments										
Males	13	6	2	1	100	49	90	44	0	
Females	2	4	3	5	29	52	21	37	1	2
All autopsied deaths	100	23	19	4	169	39	124	32	8	2

Macroscopic discoloration in the myocardium and/or histologic lesions from a recent infarct.

Table VII. Prevalence of an old infarct scar: the scar in a papillary muscle as the only old lesion and other myocardial fibrosis in persons autopsied at the pathologic and forensic departments

	Old infarct scar		Scar only in a papillary muscle		Other fibrosis		N old lesions		Isaoff data
	No.	%	No.	%	No.	%	No.	%	No.
Pathologic departments									
Males	67	51	5	4	2	2	53	40	4
Females	19	48	3	7	0		18	45	0
Forensic departments									
Males	124	61	8	4	24	12	48	23	1
Females	24	43	4	7	7	12	21	38	0
All autopsied deaths	234	54	20	5	33	8	140	32	5

Autopsy findings

A necrotic myocardial infarction was found at autopsy in 58% of the persons examined in the pathologic departments but in only 7.7% of medico-legal autopsies (Table VI). Definite or probable evidence of an acute ischemic myocardial lesion was obtained in 81% of the former cases. In medico-legally autopsied persons too, an acute infarction was demonstrated in 57%. No clear difference in sex was found in this respect in either series. A ruptured infarction was observed in 4.5% of the present autopsied persons. Rupture was more common in women (7.3%) than in men (3.6%) and more common in persons autopsied in the pathologic departments (8.2%) than in those autopsied medico-legally (1.9%). An infarct lesion in the anterior wall of the left ventricle was relatively more common in the former and a lesion in the posterior wall in the latter (Fig. 1). Of acute infarctions detected at medico-legal autopsies 81% involved at least to some extent the interventricular septum while the corresponding frequency at autopsies in the pathologic departments was 33% ($p < .001$). The location

of lesions was certainly more carefully recorded in the former than in the latter. In medico-legal cases an acute infarction was found in the posterior wall of the right ventricle as an extension of a posterior infarction in 18% of cases with a posterior lesion. A papillary muscle was involved in 42.5% of medico-legal autopsies. Of anterior infarctions 59% extended to the papillary muscle and 52% of posterior infarctions to the posterior papillary muscle.

An old myocardial infarction was found at autopsy more often in men (57%) than in women (45%) ($p < .05$) but with about a similar frequency in persons autopsied in the pathologic and forensic departments (Table VII). A similar situation was observed for the size of hearts: heavier organs in men than in women but no difference between persons autopsied in different places (Table VIII). An aneurysm in a great scar of the left ventricular wall was found in 14% of cases with an old infarction at autopsies in the pathologic departments and in 12% at medico-legal autopsies. The overall prevalence of an aneurysm was 7% of all autopsies. An

Table VIII. Heart weight in persons autopsied in the pathologic and forensic departments

	Data available	Heart weight (g)			
		Mean	S.D.	Median	Range
Pathologic departments					
Males	116	532	126	520	40-990
Females	37	439	108	406	300-650
Forensic departments					
Males	195	517	121	490	320-1 020
Females	55	436	96	430	250-660

infarct scar was equally common in the anterior and posterior walls of the left ventricle (Fig. 2). Of old infarctions in medico-legal cases 52% and of lesions in persons autopsied in the pathologic departments 51% were located at least to some extent in the myocardium of the interventricular septum. A scar in a papillary muscle as the sole old lesion was found in 5% of autopsied cases.

A fresh coronary thrombosis was recorded in 29% of autopsies from the pathologic departments and in 23% of medico-legal autopsies. The overall prevalence of an occlusion in any of the coronaries was 56% in the former and 53% in the latter, the difference not being significant. The reported frequencies of an occlusion in different coronary arteries were as follows: Autopsies in the pathologic departments: right coronary artery 32%, left anterior descending coronary artery 38% and left circumflex coronary artery 19%. Medico-legal autopsies: right coronary artery 32%, left anterior descending coronary artery 26% and left circumflex coronary artery 18%. No significant difference in sex was found in relation to the prevalence of coronary occlusions (56% for men, 52% for women). In 11% of the cases no marked stenosis was observed in coronaries. Such cases were reported more often from the pathologic (18%) than from the forensic department (7%).

DISCUSSION

The reliability of the diagnosis of registered IHD deaths appeared to be good in the present community study, which was based on a population of 550 000 inhabitants of Helsinki. For practical reasons it was not possible to apply a special methodology to the patho-anatomic investigation of hearts in all departments of pathology in the city. Since the reliability of the diagnosis of IHD death certainly is most problematic in cases of sudden death, special attention was focused according to the suggestions of the WHO (32) on the examination of medico-legal cases. As the autopsy findings in other victims were based on the routine work performed by several pathologists, the detailed comparisons with regard to the patho-anatomic findings may be regarded only as indicative of trends. Another problem in the retrospective study was caused by differences in the reliability of data of the disease history of the persons studied. It was possible to get first-hand information directly from most patients dying in hospitals, while in cases of

sudden death occurring outside hospitals the information was obtained from relatives or other persons.

Although the patho-anatomic signs of chronic heart disease, the size of the heart and the occurrence of an old infarction were similar in persons autopsied in the pathologic and forensic departments, a known symptomatic heart disease, visits to a doctor and use of digitalis were clearly less common in the latter than in the former. The discrepancy between the clinical history of a previous infarction and the autopsy finding of an old myocardial infarction was found in particular in medico-legally autopsied cases. The second-hand nature of information on the disease history in cases of sudden death may in part explain this discrepancy. On the other hand, myocardial changes in an acute infarction are visible by conventional macroscopic and histologic methods at autopsy after some 6–12 hours' duration of ischemia (12, 15). A recent detectable infarction was however rather common in the medico-legal autopsy material in persons whose fatal attack terminated rapidly, nearly instantaneously or at least within one hour of its onset. It is probable that many patients who had died suddenly outside hospitals had refused to admit their symptoms, failed to distinguish them from their usual complaints or interpreted them as originating from organs other than the heart.

A higher prevalence of acute myocardial infarction in persons autopsied in the pathologic departments than in those autopsied medico-legally fulfilled expectations. The prevalence of infarct changes in the myocardium in the latter cases was very high compared with many previous series of sudden deaths in which the frequency has varied from 8 to 28% (1, 10, 14, 30). The present frequencies are also higher than that found in a community study in Rochester, USA (27) where a recent myocardial infarction was found in 39% of all autopsied IHD deaths. A careful systematic examination of hearts by conventional methods also gives a rather high prevalence of infarct changes in sudden deaths. An acute infarction was demonstrated at autopsy in 40% of sudden deaths in the series of Titus et al. (28) and in 47% in the series of Scott and Briggs (24). The principles delineated in the WHO cooperative study (33) were followed in the investigation of the majority of the present medico-legal cases. Since no generally agreed minimum criteria for the patho-anatomic diagnosis of an acute infarction exist, the

comparison of the findings made by different workers is difficult.

The anterior location of an acute infarction tended to be most common in persons autopsied in the pathologic departments and the posterior location in those autopsied medico-legally. The arterial disease, the highest frequency of an occlusion in the left anterior descending coronary artery in the former and in the right coronary artery in the latter was in accordance with the distribution of infarct changes. Titus *et al.* (28) have reported a different distribution of infarctions in a series of sudden unexpected deaths, 53% of lesions located in the anterior wall of the left ventricle and 36% in the posterior wall. When sudden death outside hospitals was attributed to an acute infarction in the present series, the interventricular septum was involved in a high percentage of cases (81%). On the other hand, in the same medico-legal series the interventricular septum was involved in only 52% of cases with an old infarction, i.e. an infarction after which the persons had survived. The present observations suggest that the risk of sudden death is high in association with posterior infarctions and particularly with infarctions which involve the myocardium of the interventricular septum. Clinical studies have shown that dangerous bradyarrhythmic complications are common in the initial phase of an acute infarction, especially in association with inferior lesions (2, 9).

There was no difference in the size of the heart, the prevalence of an old infarction or the frequency of coronary occlusions between persons autopsied in the pathologic and forensic departments. The patho-anatomic condition of the heart before the fatal attack had probably not influenced the distribution of persons dying suddenly outside and those dying in hospitals. The prevalence of infarct scars in the present series was very similar to previous findings in a community study in Rochester (77) a series collected from several centres in Finland (18) and a Swedish series of medically unattended sudden deaths (30). A higher prevalence (69%) of old or healing infarctions was reported in a series of deaths from an acute infarction by Scott and Briggs (24). A clinical history of a previous infarction has been reported in only about 30% of persons dying suddenly (4, 6). A common finding in postmortem series is that the number of myocardial scars exceed that of clinically recognized episodes (71, 25, 30). Titus *et al.* (28) found a myocardial scar in 18% of persons in whom sudden death was the first clinical manifesta-

tion of IHD. Clinical prospective studies have shown that an unrecognized infarction is rare in patients with previous angina pectoris but more likely in patients with previous diabetes or hypertension (16).

Among the autopsied persons in the present study a history of previous angina pectoris was about equally common in both sexes. Old myocardial infarctions were however clearly more common in men, particularly in the medico-legal series. Clinical prospective studies have shown that uncomplicated angina in a population is about as frequent in women as in men but IHD in women is clearly less often complicated by an attack of infarction (11). According to Lehn (13) the heart weight ratio in healthy men: healthy women is about 1.2:1. The mean values for heart weight in men and women in the present material were related in a similar way. Although the difference in the absolute heart size was marked between series, the cardiac enlargement had obviously occurred proportionally to the same extent in both sexes.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Pension Institute, the National Research Council for Medical Sciences in Finland, the Finnish Heart Association and the Paavo Lehtinen Ahvenainen Foundation.

REFERENCES

1. Adelson, L. & Hoffman, W. Sudden death from coronary disease. Related to lethal mechanisms arising independently of vascular occlusion or myocardial damage. *J Amer med Ass* 176: C29 1961.
2. Adgey A. A. J., Allen, J. D., Geddes J. S., James R. G. G., Webb, S. W., Zuck, S. A. & Partridge, J. F. Acute phase of myocardial infarction. *Lancet* 7401 1971.
3. Armstrong, A., Duncan, B., Orr, M. F., Julian, D. G., Donald, K. W., Fulton, M., Lutz, W. & Morrison, S. L. Natural history of acute coronary heart attacks. A community study. *Brit. Heart J* 34: 67 1972.
4. Baimon, C. R. & Peterson, D. R. Deaths from coronary heart disease in persons fifty years of age and younger. A community-wide study. *New Engl. J. Med.* 268: 969 1963.
5. Chiang H. N., Perlman, L. V., Fulton, M., Ostrander, L. D. & Epstein, F. H. Predisposing factors in sudden cardiac death in Tecumseh, Michigan. A prospective study. *Circulation* 41: 31 1970.
6. Fulton, M., Julian, D. G. & Oliver, M. F. Sudden death and myocardial infarction. *Circulation*, Suppl. 4: 182, 1969.
7. Gordon, T. & Kannel, B. L. Premature mortality from coronary heart disease. The Framingham study. *J. Amer med. A. s.* 15: 1617 1971.

8. Guzman, M. A., McMahon, C. A., McGill, H. C., Jr, Strong, J. P., Tejeda, C., Restrepo, C., Eggen, D. A., Robertson, W. II & Solberg, L. A. Selected methodologic aspects of the International Atherosclerosis Project. *Lab. Invest.* 18: 479 1968.
9. Hsu J. Mechanisms of ventricular arrhythmias associated with myocardial infarction. *Amer J Cardiol* 24: 800 1969.
10. Haenen, J. P. H. Coronary death in younger persons. *Dtsch. med. Woch.* 15: 301 1968.
11. Kannel, W. B. & Feinleib, M. Natural history of angina pectoris in the Framingham study. Prognosis and survival. *Amer J Cardiol* 29: 134 1972.
12. Knight, B. Early myocardial infarction. Practical methods for its post-mortem demonstration. *J. forensic. Med.* 14: 101 1967.
13. Lehtilä, H. Normal weights of human organs. A post mortem study on cases of death from external causes. Helsinki 1971.
14. Lake, J. L. & Helpers, M. Sudden unexpected death from natural causes in young adults. A review of 275 consecutive autopsied cases. *Arch. Path.* 83: 10 1968.
15. Mallory, G. K., White, P. D. & Salcedosalgar, J. The speed of healing of myocardial infarction. A study of the pathologic anatomy in seventy-two cases. *Amer Heart J* 18: 647 1939.
16. Margolis, J. R., Kannel, W. B., Feinleib, M., Dawber, T. R. & McNamara, P. Clinical features of unrecognized myocardial infarction. Silent and symptomatic. *Amer J Cardiol* 32: 1 1973.
17. McNally, R. H. & Pemberton, J. Duration of last attack in 998 fatal cases of coronary artery disease and its relation to possible cardiac resuscitation. *Brit. med.* 1: 3: 139 1968.
18. Miettinen, M. Myocardial infarction and coronary atherosclerosis in Finland. An autopsy study covering 12 months in 1964-1965. *Acta path. microbiol. scand.* Suppl. 205 1969.
19. Nachlas, M. M. & Shmida, T. K. Macroscopic identification of early myocardial infarct by alterations in dehydrogenase activity. *Amer J Path.* 42: 379 1963.
20. Rissanen, V. T. Double contrast technique for post-mortem coronary angiography. *Lab. Invest.* 23: 517 1970.
21. — Occurrence of coronary occlusion in cases of coronary death and accidental death. *Adv. Cardiol.* 4: 99 1970.
22. Romo, M. Factors related to sudden death in acute ischaemic heart disease. A community study in Helsinki. *Acta med. scand.*, Suppl. 547 1973.
23. Sample census of population in Helsinki 1965. Published by the Statistical Office of the City of Helsinki. Helsinki 1968.
24. Scott, R. F. & Briggs, T. S. Pathological findings in pre-hospital deaths due to coronary atherosclerosis. *Amer J Cardiol* 29: 782, 1972.
25. Slevens, J. Myocardial infarction. Clinical features and outcome in three thousand thirty-six cases. *Acta med. scand. Suppl.* 406, 1963.
26. Siltanen, P. The ischaemic heart disease register as frame for preventive measures. *Adv. Cardiol.* 8: 214 1972.
27. Spielerman, R. E., Brandenburg, J. T., Asher, R. W. P. & Edwards, J. E. The spectrum of coronary heart disease in a community of 30 000. A clinicopathological study. *Circulation* 25: 57 1962.
28. Titus, J. L., Ounonen, H. A., Nobrega, F. T. & Connolly, D. C. Sudden unexpected death as the initial manifestation of ischaemic heart disease. Clinical and pathological observations. *Amer J Cardiol* 26: 662 1970.
29. Vedin, J. A. Hjärtinfarkt i Göteborg 1958-1970. Dödsfall, riskfaktorer och prognostiska faktorer under två års uppföljning av patienter som överlevt akut besvärstiden. Göteborg 1974.
30. Wiklund, H. Medically unattended fatal cases of ischaemic heart disease in a defined population. *Acta med. scand. Suppl.* 524 1971.
31. WHO. Arterial hypertension and ischaemic heart disease. Preventive aspects. Report of an expert committee. *Wld Hlth Org. techn. Rep. Ser.* 231: 1 1962.
32. — Pathological diagnosis of acute ischaemic heart disease. Report of a WHO Scientific Group. *Wld Hlth Org. techn. Rep. Ser.* 441 1970.
33. — The pathological diagnosis of acute myocardial infarction. Preliminary results of a WHO Cooperative Study. *Bull. Wld Hlth Org.* 48: 23 1973.
34. — Working Group of Ischaemic Heart Disease Registers. 29 June-1 July 1970. Copenhagen. Report of the 4th working group with revised operating protocol. Regional Office for Europe. WHO/EURO 5010 (4). Copenhagen 1970.

ISOLATED VALVULAR AORTIC STENOSIS

Clinico-pathological Findings in an Autopsy Material of Elderly patients

Johan A. Andersen, Birgit Fischer Hansen and Kjeld Lynsborg

*From the Institute of Pathology and the Medical Department
Sundby Hospital, Copenhagen, Denmark*

Abstract In a review of 2357 autopsy reports from general hospital 20 patients, aged 65 or above, have been found to have severe isolated valvular aortic stenosis. The diagnosis was made or suggested clinically in only 5 cases. The clinical findings were often atypical, e.g. the BP amplitude was above 50 mmHg in 9 patients and 4 had systolic BP of 180 mmHg or more. Left ventricular hypertrophy as evaluated by ECG was however present in all examined cases but one. The main cause of death was cardiac in 17 cases of whom 2 died suddenly and 4 following non-cardiac surgery. At autopsy significant coronary arteriosclerosis was found in all but 2 patients. In 9 patients acute myocardial infarction was demonstrated—in cases only through a microscopic enzyme test (Nifro-BT method).

Complete autopsy of the heart was performed in all cases. The heart was weighed and the outer dimensions were measured transversely along the aortic coronary and longitudinally from the latter to the apex cordis. The ostia of the heart were explored and the sizes estimated. The type of IVAS was evaluated according to the criteria of Posselt (12). The coronary arteries and later the heart were cut open. The thickness of the myocardium of the left and the right ventricle was measured. The myocardium was then cut and examined for acute myocardial infarction (AMI) and/or fibrosis. The extent of diffuse arteriosclerotic changes in the coronary arteries was graded (0-3). Localized, severe stenosing arteriosclerosis as well as subintimal hemorrhages and thrombi were also recorded.

In recent years the beds of medical departments have increasingly been used for treatment of elderly patients. Although heart diseases in this age group are mainly of ischemic etiology rarer causes of heart failure are naturally also met. We have recently noticed that a number of elderly heart patients at autopsy were found to have isolated valvular aortic stenosis (IVAS). The purpose of this paper is to describe the clinical and pathological findings in these cases.

MATERIAL AND METHOD

The material consists of 20 cases (9 men and 11 women) of IVAS aged 65 or above (range 65-89). The average age for men was 74.6, for women 77.6 years. The age and sex distribution appears in Table I. These cases were found by examining autopsy reports from the Institute of Pathology, Sundby Hospital, during Jan 1968 - April 1972. The Institute serves a population of approximately 120 000 persons. During this period 2357 autopsies were performed. Only cases in which it was clearly indicated that the aortic ostium was narrowed and just allowed the passage of finger (grade 1) or less (grade 2) were included. All cases with changes localized to valves in mitral or tricuspid ostium were excluded.

RESULTS

Clinical findings (Table I). Fourteen of 20 patients died in a medical department. Of the remaining 6 patients 4 were admitted to a surgical ward, one to a gynecological ward and one to an ear, nose and throat department. Two of the 20 patients died within 1 hour after admission, one during the first day, 5 within the first week and 8 within 3 weeks and 4 21-30 days after admission.

Ten patients had had symptoms attributable to aortic disease for 1 year or longer, 5 for less than 1 year. Information about 5 patients was insufficient. Fifteen patients had dyspnea prior to hospitalization during which they died. In 3 patients no dyspnea prior to admission was noted, information about 2 was insufficient. Ten patients had precordial pain prior to admission, 6 did not have. In 4 cases it is not known whether they had precordial pain or not. Three patients had syncope. In 7 it was not clear whether they had syncope or not. Aortic stenosis (AS) was diagnosed clinically in 4 cases and considered in 1 case. In 15 cases the diagnosis of AS was not mentioned. Systolic murmur of typical location

In our experience LVH as seen in the ECG was present in 17 of 19 cases examined (1 of the 2 cases who did not show LVH had only standard leads taken). Although the presence of LVH in the ECG should give support to the suspicion of AS it should be remembered that it is difficult to know whether the established criteria for LVH also apply to the elderly and that other causes of LVH e.g. hypertension are present particularly in the elderly.

Useful X-ray examination was only made in a few cases as mentioned due to the patients' clinical condition. Enlargement of the left ventricle and dilatation of the ascending aorta are signs of aortic valvular stenosis. These signs were also found in the majority of our cases. In the elderly they are diagnostically less helpful as they are commonly seen in other diseases such as arterial hypertension and arteriosclerosis of the aorta and the coronary arteries.

This study does not allow any conclusion as to the prognosis of severe IVAS. Thirteen of the 20 patients died; however 2 of them suddenly directly as a consequence of their heart disease. In 4 other cases who died following operation IVAS played a significant role. Thus in 17 of 20 cases IVAS was the major cause of death although a number of co-factors existed. Among the findings as such the most noteworthy is perhaps the extent of

osis in coronary arteries. Only 2 of the 20 patients did not have moderate to severe diffuse arteriosclerosis. Almost occluding localized stenosis of the coronary arteries was, moreover, found in 11 patients.

These findings are in agreement with those of Roberts et al. (13). In our material we thus find nothing to support the view that patients with IVAS often show very little arteriosclerosis of the coronary vessels nor do we find a basis for the theory that the degree of IVAS is inversely proportional to the degree of coronary arteriosclerosis as mentioned by Horan and Barnes (7). The incidence of AMI is high in our material (45%). Kumpe and Bean (8) found in a similar material an incidence of 12.1%. Robert et al. (13) 4% and Bergeron et al. (3) only 3%. As in the two latter reports we find that the myocardial infarctions were non-thrombotic: two-thirds of the infarctions in the material of Kumpe and Bean (8) were of the same type. These findings are in clear contrast to the previously accepted opinion that AMI is seldom seen in patients with IVAS (9).

The high incidence of myocardial infarction found in this study is partially due to the use of the Nitro-BT method (1) as myocardial infarction in 2 cases was not seen by ordinary gross examination but was demonstrated by the former method. As regards the pathogenesis of IVAS the distribution according to types in our material is in agreement with the findings of Pomerance (12). It is thus our conclusion that IVAS and death due to heart failure are common among elderly patients. Severe and obstructing arteriosclerosis of the main coronary arteries is found in the majority of these patients. If valve surgery is considered in this age group, visualization of the coronary arteries should be contemplated since operative mortality among unselected elderly patients would be expected to be high.

REFERENCES

1. Andersen J. A. & Hansen, B. F. Isolated acute myocardial infarction of papillary muscles of the heart. *Brit. Heart J.* 35: 781 1973.
2. Bedford, P. D. & Caird, P. I. Valvular disease of the heart in old age. p. 60. Churchill, London 1960.
3. Bergeron J. Abelmans W. H. Vazquez-Milan, H. & Ellis, L. II Aortic stenosis—clinical manifestations and course of the disease. *Arch. Intern. Med.* 94: 911 1954.
4. Finegan R. E., Ghirelli R. E. & Harrison D. C. Aortic stenosis in the elderly. *New Engl. J. Med.* 281: 1261 1969.
5. Hancock E. W., Madison, W. M., Proctor M. H., Abelmans, W. H. & Starkey G. W. II Aortic stenosis of no physiologic significance. *New Engl. J. Med.* 258: 305 1958.
6. Heine, W. L., Sackett, C. F. & Serber W. Electrocardiographic criteria of left ventricular hypertrophy. *Amer. J. med. Sci.* 224: 424 1952.
7. Horan M. J. & Barnes A. R. Calcareous aortic stenosis and coronary artery disease. *Amer. J. med. Sci.* 215: 451 1948.
8. Kumpe C. W. & Bean, W. B. Aortic stenosis: A study of the clinical and pathologic aspects of 107 proven cases. *Medicine* 27: 139 1948.
9. Levine, S. A. Clinical heart disease. Saunders, Philadelphia 1945.
10. Møller C. Aortic stenosis and the so-called rheumatic valvular disease in a post mortem material. *Acta med. scand.* 156: 241 1956.
11. Pridman, V. & Hamet, A. Possibility of specific diagnosis of left ventricular hypertrophy. *Cor et vas (Praga)* 11: 1 1969.
12. Pomerance A. Pathogenesis of aortic stenosis and its relation to age. *Brit. Heart J.* 34: 569 1972.
13. Roberts, W. C., Perloff J. K. & Constantino T. Severe valvular aortic stenosis in patients over 65 years of age. *Amer. J. Cardiol.* 27: 497 1971.

THE FREQUENCY OF SECONDARY HYPERTENSION

Karine Bech and Tage Hilden

From Medical Department C, Dialektskeftstiftelsen, Frederiksberg, Denmark

Abstract: In a series comprising 482 patients with hypertension requiring treatment 79% had to be classified as essential hypertension. Bilateral renal disease was found in 9%, unilateral renal disease in 3.3% but only one patient underwent surgery. Renal artery stenosis was found in 24 patients (5%), but only 5 (1%) were operated on. Two cases of primary hyperaldosteronism and one of pheochromocytoma were found, in all three surgical intervention was successful. Oral contraceptives had caused the elevated BP in 7 patients (1.5%). It is emphasized that the frequency of curable hypertension is still very low and this should be taken into account when routine examination of patients with hypertension requiring treatment is discussed.

The majority of cases of elevated blood pressure must still be classified as essential hypertension also called primary hypertension. In a certain group however the hypertension can be attributed to detectable conditions and is therefore termed secondary hypertension. The finding of such conditions is particularly important, since the underlying cause and thereby the hypertension may in some cases be remedied by surgical intervention. This is of course much to be preferred to prolonged drug therapy which in some cases is not well tolerated.

Considering the high incidence of hypertension requiring treatment in the general population the study of hypertensive patients with the object of revealing specific etiological factors must be considered a rather demanding task and is consequently today a much discussed topic. Such investigations must, of course, be related to the incidence of secondary—including curable—hypertension. Therefore we should like to report on our experience in a group of hypertensive patients.

PATIENTS AND METHODS

The series includes 482 patients: 272 males and 210 females, with elevated BP examined in our unit over the period 1958-72, with the object of revealing specific etiological factors. The age distribution was as follows: 19% <40, 65% 40-59 and 16% ≥60 years of age.

Most of the patients were referred because of hypertension and, consequently the series must be considered to be selected. The criterion for submitting the patients to an etiological study was that the hypertension was considered to require treatment along the lines established in Table 1. The severity of the hypertension was assessed on the basis of the organic changes. Applying the Keith-Wagener classification of hypertensive retinopathy 69% had grades I and II, 14% grade III without fresh exudates, 11% grade III with fresh exudates and 5% had grade IV. The serum creatinine level was normal in 72% (1.4-2.9 mg/100 ml), in 25% and higher than 3.0 mg/100 ml in 3%.

Since the value of a series which is intended to clarify the incidence of secondary hypertension depends very much on consistent progression of study the procedures will be described in detail.

Urography was performed in 464 patients (96%), i.e. not carried out in 18 patients. The reasons for omitting this investigation were as follows: two patients died suddenly, 14 had severe uraemia, one patient was allergic to contrast media, and in one woman who became nonretentive after having stopped taking oral contraceptives the examination was simply for gotten.

During the first few years rapid sequence urograms were taken followed by tomograms. During recent years we have applied excretion urography. As supplement we previously used renal angiography later isotope renography. Angiography was performed in 32% of the cases and renography in 44%. These supplementary examinations were not made if the patient died shortly after admission or if pronounced uraemia was present. They were also omitted in patients with only one kidney and in very old or sick patients who could not tolerate surgery.

Serum potassium was determined in all cases in 79% before commencement of treatment. (A more de-

Table I. Indications for antihypertensive therapy

Organic changes

Ocular	Hypertensive retinopathy grade IV
	Hypertensive retinopathy grade III with fresh exudates
	Hypertensive retinopathy grade II in younger patients
Cardial	Congestive heart failure
	Angina pectoris
	Past myocardial infarction
	Cardiac enlargement
	Pathological ECG
Cerebral	Acute encephalopathy
	Past stroke (suitable cases)

Permanent diastolic hypertension at the age of

	Diastolic BP (mmHg)
<40	≥100
40-49	≥105
≥50	≥110

tailed analysis of the potassium values found will be given later.)

During the first part of the study screening for pheochromocytoma was carried out by means of the regitine test, which was soon replaced by determination of catecholamines and/or examination of 24-hour urine specimens for metanephric acid content. These tests were made in 378 patients (78%). In the remaining 104 patients the tests were omitted in 38, in whom the diagnosis of pheochromocytoma could be clearly excluded or would be of no clinical interest. During the retrospective study however we realized that in 66 patients the test should have been done routinely although in these cases there was no clinical evidence of pheochromocytoma (it will be shown later in this paper that routine examination did not reveal

Table II. Distribution of patients according to etiology

	N	%	Surgically treated
Essential hypertension	382	79.1	
Bilateral renal disease	45	9.3	
Unilateral renal disease	16	3.3	1
Renal artery stenosis	24	5.0	5
Special cases	5	1.0	
Primary hyperaldosteronism	2	0.4	2
Pheochromocytoma	1	0.2	1
Oral contraceptives	7	1.5	
Total	482	100.0	9

Table III. Incidence of renal diseases

Diagnosis

Bilateral renal disease

Chronic glomerulonephritis	14
Chronic pyelonephritis	16
Chronic interstitial nephritis	9
Diabetic nephropathy	2
Polycystic kidneys	3
Medullary sponge kidney	1

Unilateral renal diseases

Congenital aplastic kidney	6
Pyelonephritic contracted kidney	6
Hydronephrosis	2
Renal cyst	1
Renal dysplasia	1

any case of pheochromocytoma, the only case was found by X-ray.)

Finally we tried to palpate the femoral artery in all the patients—at least in those below the age of 40. Young females were questioned about their use of oral contraceptives. We have not made any systematic inquiry as to intake of liquorice.

RESULTS

The classification of the 482 patients is given in Table II. In 79% the hypertension had to be classified as essential.

Bilateral renal disease was found in 45 patients and unilateral renal disease in 16 (Table III). Only one patient underwent surgery as 48-year-old female with congenital aplastic kidney but the outcome was poor. All other patients received drug treatment.

Renal artery stenosis was found or strongly suspected in 24 patients (5%). In 9 cases we were, however, unable to confirm the finding of renovascular hypertension. Nine of the remaining patients received drug treatment firstly because this proved easily manageable and effective (6 patients), and secondly because surgery was contraindicated (3 patients). Five patients underwent surgery: three became normotensive, one improved and in one patient the condition was unchanged. Finally one female with symptoms of fibromuscular hyperplasia is still under observation; her BP became normalized spontaneously.

In 5 patients (classified as special cases in Table II) a specific endocrine etiology was suspected. One patient with hypertension of moderate severity had a very high serum renin level and

Table IV Evaluation of cases with low serum potassium level

	Un-treated	Receiving diuretics
Response to potassium supplement	5	5
Response to spironolactone	1	3
Renin/aldosterone determination		
Clinical course	5	1
Autopsy	6	3
Primary hyperaldosteronism	2	6
Other endocrine disorders possible	2	0
Total	1	1
	20	21

aldosterone excretion without any demonstrable renal cause. In three patients changes in the renin and aldosterone levels were found which were incompatible with primary hyperaldosteronism, but which might indicate the presence of another mineral corticoid metabolic disorder. In all these cases the hypertension was adequately controlled by drug treatment. One patient had Turner's syndrome.

Two cases of primary hyperaldosteronism were found and in both the hypertension was relieved by surgery. Since primary hyperaldosteronism must be suspected in any case of hypokalaemia, we shall describe the cases in which the serum potassium levels were below 3.5 mEq/l, i.e. 20 untreated patients and 21 patients who received diuretics at the commencement of the study. With the object of further elucidating the cause we have evaluated the following features: pronounced effect of potassium supplement, lack of effect of spironolactone treatment, determination of plasma renin and aldosterone production. The remaining features were assessed on the basis of the clinical course or at autopsy (Table IV). Apart from the two cases described above primary hyperaldosteronism can in our opinion be excluded in the remaining patients.

One case of pheochromocytoma was found and operated on with favourable results. No cases of Cushing's syndrome were found.

In 7 cases oral contraceptives were the cause of the hypertension. The influence of such drugs being proved during several observation periods with and without oral contraceptives. In all cases the hypertension was of moderate severity only.

Table V Classification of the etiology of the hypertension in three series

	Kennedy et al. (4)	Hood and Björk (2)	Present series
No. of pts.	750 (5%)	683 (5%)	482 (5%)
Essential hypertension	72.6	39	79.3
Bilateral renal disease	7.7	8.9	2.3
Unilateral renal disease	9.5	1.9	3.3
Renal artery stenosis	6.1	13.7	5.0
Primary hyperaldosteronism	0.3	0.3	0.4
Cushing's syndrome	—	0.1	—
Coarctation of aorta	—	1.0	—
Oral contraceptives	—	—	1.5
Other cases	—	24.4	1.0
Incompletely examined	3.7	9	—

In two younger patients the consumption of liquorice was excessive but the increase in BP was not remedied when the intake of liquorice was stopped. No cases of coarctation of the aorta were found.

DISCUSSION

A few surveys of the same nature as our study have been made. Our results and those from two earlier studies are summarized in Table V. However it is extremely difficult to compare these series since both the diagnostic criteria and the selection of the patients differ. Our results agree well with those found by Kennedy et al. (4). In the material presented by Hood and Björk (2) the incidence of essential hypertension is remarkably low. However the latter series comprised two groups, unclassifiable (24%) and incompletely investigated (9%) which probably included some cases of essential hypertension. Furthermore, this material might well be specially selected in that several of the patients must be presumed to have been referred to the Gothenburg Centre because of special diagnostic problems. The age distribution in the series will also exert a certain influence because as is known from various surveys (1, 7, 9), the causes of the hypertension will often be specific in younger age groups. Platt (8) found secondary hypertension in 70% of patients below the age of 35.

Although it is reasonable to presume that a specific etiology might be detected or suggested in about 20% of the cases when the series

prises hospitalized patients with hypertension requiring treatment, it does not follow that radical treatment will be possible in such cases.

Bilateral renal disease is present in about half the cases of secondary hypertension (8-9%) and in these cases drug therapy is the treatment of choice. In our material 14 patients had chronic glomerulonephritis which must be considered the cause of the hypertension. In the remaining patients of this group the etiological influence of the renal disease is doubtful since the concurrent appearance of essential hypertension and renal disease cannot be definitely excluded. This is particularly the case in patients with suspected chronic pyelonephritis.

The reported frequency of unilateral renal disease as a possible cause of the hypertension varies greatly (2-10%) and this is no doubt accounted for by the uncertainty with respect to the importance of this etiological factor. In the 1940s a number of cases were reported in which surgery had had favourable effects but it appeared later that over a longer period the results were disappointing (10) a good deal of scepticism prevails on this point. In a series reported by Kirkendall et al (5) a permanent fall in BP was obtained in only one patient of 10 operated upon for unilateral renal disease.

In 16 of our cases (3.5%) unilateral renal disease was considered to be the possible etiological factor but in only one case was surgery carried out and with a negative result. The remaining patients received drug treatment. However with the examination technique now available, in particular unilateral renal vein catheterization with renin determination, it will no doubt be possible to predict the result of surgical intervention more accurately. When evaluating our series retrospectively we find that such examinations should have been done in at least 4 cases, although in two of them surgery was definitely contraindicated.

Renal artery stenosis as the cause of hypertension has been of current interest during the last 10-15 years and is today the most frequent cause of surgically treatable hypertension. Good results have been obtained in many of the cases operated on, but the selection is quite decisive. Narrowing of the renal arteries found by angiography is rarely a sufficient indication for operation, such changes in the lumen are very frequent in hyper-

tensive patients and also in normotensive persons, particularly after the age of 50. Consequently when selecting patients for surgery the decision should also be based on other examinations of which unilateral renin determination is the most important. However in many patients in particular the elderly drug treatment should be preferred.

The incidence of renal artery stenosis in a series of hypertensive patients varies greatly e.g. depending on the value attached to the angiographic findings. In our patients we have stated renal artery stenosis to be the possible cause in 74 (5%) but only 5 were operated on (1%). With the indications which are commonly followed today the frequency of operated renal artery stenosis would range between 1 and 2% in a group of hypertensive patients.

We encountered two cases of primary hyperaldosteronism and one with pheochromocytoma. The incidence of these rare diseases in our patients agrees well with that found in other series (Table V). It should be emphasized that no cases of pheochromocytoma were revealed by the usual screening methods; our only case was found by X-ray.

Finally we had 7 cases in which withdrawal of oral contraceptives normalized the high BP. As regards this problem reference is made to other reports (3, 6).

As stated in the introduction, examination of patients with hypertension with a view to revealing any specific etiology is extremely important and some authors have maintained categorically that such extensive examinations should be performed in every case of hypertension requiring treatment. However such efforts should also be reasonably related to the available possibilities for radical treatment. In our series this was the case or had been attempted in 9 patients of 482 i.e. in 2%. Hence it must be realized that curable hypertension still represents a very limited proportion of our patients with hypertension.

REFERENCES

1. Breckenridge A., Preger L., Doherty C. T. & Laws J. W. Hypertension in the young. *Quart. J. Med.* 36: 549 1967.
2. Hood, B. & Björk, S. The diagnosis essential hypertension. *Acta med. scand.* 181: 63 1967.

3. Jensen, H. Æ., Lund, J. O., Moshech, N., Hilden, T., Dømkjer Nielsen, M. & Giese, J. Orale kontraseptiva og hypertension. II. Under søgtes over blodtryksniveau og renin-angiotensin-systemets forhold ved p-placering. *Ugeskr. Læg.* In press 1974.
4. Kennedy, A. C., Luke, R. G., Briggs, J. D. & Stirling, W. B. Detection of renovascular hypertension. *Lancet* 2. 963 1965.
5. Kirkendall, W. M., Fitz, A. E. & Lawrence, M. S. Renal hypertension: Diagnosis and surgical treatment. *New Engl. J. Med.* 276. 479 1967.
6. Lund, J. O. & Jensen, H. Æ. Orale kontraseptiva og hypertension. I. En oversigt. *Ugeskr. Læg.* I press 1974.
7. Ooi, B. S., Chan, B. T. M., Toh, C. C. S. & Khoo, O. T. Causes of hypertension in the young. *Brit. med. J.* 3. 744 1970.
8. Platt, R. L. Severe hypertension in young persons. *Quart. J. Med.* 17. 63 1948.
9. Singh, S. P. & Page, L. B. Hypertension in early life. *Amer. J. Med. Sci.* 253. 31 1967.
10. Smith, H. W. Hypertension and aortic disease. *Amer. J. Med.* 4. 724 1948.

Table I. Results of fine needle aspiration biopsy with two different staining techniques

Pat. no	Sex.	Age (y)	Palpable neck abnormality	May-Grünwald-Giemsa		
				Malignant C-cells	Normal cytology	Alkaline Congo amyloid positive
Group A						
1	♀	32	+	+		+
2	♂	57	+	+		+
3	♀	22	+	+		
4	♀	54	Absent		+	
5	♂	58	+	+		+
6	♂	59	Absent	Biopsy material insufficient for evaluation		
7	♂	33	+	+		+
8	♀	35	+		+	
9	♀	22	Absent	Biopsy material insufficient for evaluation		
10	♂	22	Absent	+		+
Group B						
11	♀	72	+	+		Not examined
12	♀	77	+	+		+
13	♀	36	+	+		
14	♂	71	+	+		+
15	♂	51	+	+		+
16	♂	46	+	+		+
Group C						
17	♂	72	+	+		
18	♂	54	Absent	Biopsy material insufficient for evaluation		
Total:			13/18	13/15	2/15	9/14

Hazard et al. (7) and Williams et al. (25). The other 2 were proved carriers of the Sipple genome, had high levels of S-CT as well as pheochromocytoma but refused surgery for MCT (nos. 6 and 8). patients are divided into three groups A, B and C (Table I).

Group A. New cases of hereditary MCT. Three families with Sipple's syndrome (22), altogether 47 members were screened prospectively for MCT and pheochromocytoma. This screening program includes 1) careful clinical examination, 2) thyroid scintiscan using ^{123}I and/or $^{99\text{m}}\text{Tc}$, 3) determination of S-CT both (a) basal level and (b) the response to induced hypercalcaemia, using our radioimmunoassay method for human S-CT (1), 4) determination of diamine oxidase activity (E.C.1.4.3.6. histaminase) according to Tufvesson and Tryding (23), and 5) fine needle aspiration biopsy of all palpable thyroid nodules or lymph nodes of the neck ("palpable neck abnormality" in Table I) (In 4 patients (nos. 4, 6, 9, 10) aspiration biopsy from the thyroid was attempted although there were no palpable neck abnormalities.) In these families 10 new patients with subsequently verified MCT were found (nos. 1-10).

Group B. New sporadic case (seen in the Outpatient Clinic for thyroid disorders). Six patients without known heredity for Sipple's syndrome were examined because of nodular goitre (nos. 11-13) or neck lymphoglandular metastases from primary tumours, the nature and localization of which was unknown (nos. 14-16).

Group C. Cases of earlier operated sporadic MCT. Two

patients were previously hemithyroidectomized for sporadic MCT. One presented with large mass in the neck (no. 17), the other had no palpable abnormality but raised S-CT and microscopically there was insufficient radicality of the surgical specimen (no. 18). Fine needle biopsy was performed from beneath the scar of the previous thyroid operation. These patients were examined by us to verify suspected metastases of MCT.

Controls. To study the specificity of amyloid in MCT we examined 60 fine needle aspiration biopsies from 30 consecutive patients with miscellaneous types of goitre other than MCT seen as outpatients in our clinic for thyroid disorders. Smears from each patient were stained with May-Grünwald-Giemsa and with alkaline Congo.

METHODS

The instruments used for the aspiration biopsies were either the one-hand syringe of Franzén et al. (6) or disposable plastic syringe (10 ml) with an adaptor (Carasco, Sweden). Both are so constructed that an excellent grip for one-handed manipulation during the biopsic procedure is possible. The *puncture technique* has been thoroughly described previously (19). During maximum aspiration the needle is moved back and forth in the puncture channel for a few seconds. Prolonged aspiration often causes too heavy an admixture of blood. Care must be taken to discontinue the aspiration before the needle is withdrawn, otherwise the aspirate will spread on the walls of the syringe.



Fig 1 MCT cells as they appear in wet-fixed aspirates stained with hematoxylin-eosin. 400.

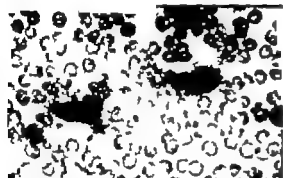


Fig 2 Two MCT cells with the aggregated, "dendritic cell" appearance which is common in some tumours. May-Grünwald-Giemsa. 400.



Fig 3 The transular cell in the centre is typical red-granulated MCT cell. Cell to the left below presents the bluish-gray cytoplasmic condensation, though to be intracellular amyloid. May-Grünwald-Giemsa. 1000.



Fig 4 Some aspirate specimens of MCT present monomorphous and non-specific populations of small tumour cells which the diagnostic red-granulated cells (giant cell in centre) have to be sought for. May-Grünwald-Giemsa. 400.

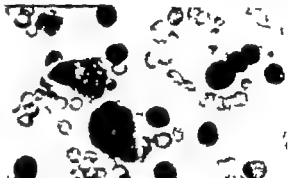


Fig 5 MCT cells as they appear in dry fixed aspirates stained with May-Grünwald-Giemsa. Note the single monogranulated red-granulated cell and the nuclei seemingly "about-to-leave" the cytoplasm in some of the small cells, feature rather typical of these APUD tumour cells in smears. 400.



Fig 6 Survey with many cells characteristic of MCT. May-Grünwald-Giemsa. 100.



Fig 7 Aspirate from MCT stained with Congo red for amyloid viewed in non-polarized light to the right and with crossed polaroid filters to the left. A large amyloid deposit in the centre. 100.

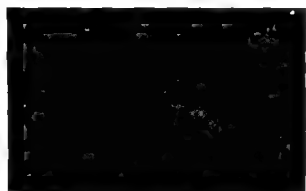


Fig 8 Smears should be screened under crossed polarization filters for the presence of the green luminescence of amyloid. Congo red. 100



Fig 9 T-lymphocyte staining lump of amorphous substance thought to be amyloid. May-Grünwald-Giemsa. 1000.



Fig 10 Askanazy cells from a Hurthle cell adenoma, easily distinguished from MCT cells by their dense blue granulation. May-Grünwald-Giemsa. 1000.



Fig 11 MCT cells (left) and pheochromocytoma cells (right) in smears from patient 1. May-Grünwald-Giemsa stained aspirates these tumours yield virtually identical cytological pictures. 400.



Fig 12 Chemodectoma cells difficult to distinguish from MCT cells. May-Grünwald-Giemsa. 1000.

and will be difficult to expel. The aspirate is carefully smeared out on one or more glass slides, the specimens for staining according to May-Grünwald-Giemsa being air-dried, the others immediately fixed in 80% alcohol for staining with hematoxylin-eosin and alkaline Congo (17). Some smears were stained with toluidine blue and eosin alone. It is possible to destain old slides with acid methanol and restain with alkaline Congo, which was done in our first cases of hereditary MCT and in the sporadic cases where the diagnosis of MCT was not primarily suspected. In these slides as well the amyloid deposits showed the typical green birefringence in polarized light. Some patients were biopsied several times, others only once.

Histological sections from the tumours were stained with hematoxylin-eosin, van Gieson and Alkaline Congo

RESULTS

Characteristic morphology of MCT cells (May-Grünwald-Giemsa staining)

Cell size and shape Most tumour cells appeared larger than the normal thyroid follicular cells, but all sizes were represented up to multinuclear giant cells (Figs. 1-4). Quite often the cells were spindle-shaped or triangular with dendritic extensions. This cell shape was very characteristic and of high diagnostic significance which can be seen also in histological sections, as was pointed out by Tateishi et al. (70) (Fig. 2).

Cytoplasm. The rather abundant cytoplasm appeared amphophilic and structureless in most cells but in about 5-15% of the total cell population a distinct red granulation was seen (Figs. 2, 3). The granules were usually rather coarse but sometimes fine and dustlike. In a few cells the granulation appeared as a diffuse red staining in part of the cytoplasm.

The granules did not take stain with eosin alone and showed weak metachromasia with toluidine blue. In the terminology of MGG staining they may thus be defined as *azurophilic* (of the primary granules of promyelocytes) and not as *eosinophilic*. This explains why this type of granulation is not distinct in smears stained with hematoxylin-eosin, in which this eye-catching detail of the MCT cytology is lost.

The granulated cells had many specific features in common with the other cells of the population. They never appeared as a fundamentally separate type of cells but rather as differentiation extremes within the variation spectrum of MCT cells (iii) be compared e.g. with the fraction of melanin-granulated cells in fine needle samples from malignant melanomas.

In some carcinomas the granulated cells were rare

and the smears consisted mainly of non-specific tumour cells with very little cytoplasm (Fig. 4 patient 10).

Nucleus A most characteristic high proportion of binucleate cells in multinucleate cells as well were frequent and were nearly always eccentrically placed in the cell and were dark and sharply outlined. Large and distinct nucleoli were present. Nuclear pleomorphism was not marked. Mitoses were frequent. In most aspirates the tumour cells were isolated and in groups the most characteristic cell was ever often being isolated. It was not difficult to distinguish the so-called Askanazy or Hürthle cells from the MCT tumour cells. The Askanazy cells had a dark-blue dustlike granulation restricted to part of the "smoke-grey" cytoplasm (Fig. 10) (16).

Amyloid In many smears there were amorphous lumps of a substance stained red or violet with May-Grünwald-Giemsa and red with hematoxylin-eosin. Usually the amyloid was located outside the cells but sometimes it seemed to be present also intracellularly (Fig. 5, 9). With alkaline Congo the amyloid was highly refractile and showed a common light microscopy (Fig. 7). In polarized light the amyloid showed the characteristic green birefringence (Figs. 7, 8). Material sufficient for diagnostic evaluation with alkaline Congo stain was obtained in 14 patients, 9 of them were positive for amyloid (Table I). In none of our controls could we find extra- or intracellular lumps of amorphous substance with amyloid criteria. Therefore we conclude that MCT amyloid does represent amyloid and not only denatured thyroglobulin.

To summarize the whole tumour cell population looked rather alien in normal thyroid cell smears from an aspiration biopsy and appeared clearly neoplastic (Fig. 6). The characteristic cell was triangular with one or more nuclei eccentrically placed. The rather abundant cytoplasm had a bright red granulation and between the cells and cell groups sometimes also intracellularly there were lumps of amyloid.

Histology

All slides from the operative specimens were reviewed, specially with regard to the occurrence of amyloid deposits.

In all but one patient (no. 10) there were varying

amounts of amyloid. This patient had multiple tumours of peanut size in both lobes. The tumours were composed of uniform cells with rounded nuclei and rather scanty cytoplasm. The cells were arranged in trabeculae or in small follicular structures. The tumour stroma was scanty and no amyloid could be seen in the histological sections. Serial sectioning was not performed. *Fine needle aspirates* from this patient, however, showed a few minute deposits of amyloid. The smears were dominated by small non-specific tumour cells with only few single larger red-granulated cells (Fig. 4). Thus in this case cytology was more informative than histology. The MCT diagnosis was further confirmed by the raised basal S-CT and the elevated response to induced hypercalcaemia.

Diagnostic results

Material sufficient for diagnostic evaluation was obtained in 15 patients whereas the cell yield was insufficient for diagnosis in 3 (Table 1). In these three patients (nos. 6, 9 and 18) there were no palpable abnormalities to guide the puncture needle.

Correct diagnosis was established in 13 (87%) of the 15 patients with adequate biopsies. It should be noted that one of these was the result of a "blind" puncture of the thyroid region without a convincing alipatory target (patient 10). Thus 2 of 15 patients were wrongly diagnosed. However in one of these patients (no. 4) the tumour was so small that the gland was considered normal by two clinicians experienced in thyroid disorders. The other of them (no. 8) had a soft nodular goitre in which the tumour obviously was hiding. In this patient the diagnosis has not been verified by surgery and histopathology but she belongs to a Sipple family and has been operated upon for pheochromocytoma, thus being a certified Sipple gene carrier. Repeated controls of S-CT have shown a successive rise from normal to definitely pathological levels. Therefore we believe this patient to have multiple small MCT foci which have been missed by the needle.

DISCUSSION

An early and specific diagnosis of MCT is of considerable clinical consequence. This tumour is probably more common than is usually thought, but its growth is sometimes slow and may therefore conceal the neoplastic nature of the condition. The combination of MCT with bilateral pheochromocytomas in Sipple's syndrome may cause unexpected cardio-

vascular emergency situations when general anaesthesia and operation are attempted. This emphasizes the need for an easy and reliable method of preoperative diagnosis of this tumour.

At present we are trying several methods for this purpose. In this paper the attention is drawn to the rather specific cytological morphology of MCT in fine needle aspirates.

Like other authors in Scandinavia (5, 16) we have long had the attitude that no examination of a thyroid lesion is complete without a fine needle biopsy. The subsequent evaluation of the results (27) has shown this policy to be justified: even if the cytological diagnosis of follicular thyroid carcinoma is very difficult (19).

The diagnostic problem as to MCT is different. This tumour yields a cytology with characteristic features which usually permit the specific diagnosis: a somewhat asymmetrical tumour cell with one or more nuclei eccentrically positioned, many of them having a distinct bright red granulation. The amyloid deposits with a green birefringence in polarized light—also well visible in May-Grünwald-Giemsa and hematoxylin-eosin staining—may finally secure the specific diagnosis.

Once the specific morphology of MCT cells had been recognized, similar cells interpreted as C-cells were detected in non-neoplastic thyroid tissue especially in thyrotoxicosis (12). However the few and isolated C-cells seen in such conditions never presented a problem of differential diagnosis in relation to MCT.

Why do some or the majority of the MCT tumour cells appear without the characteristic granulation? In several other amine-containing cells with endocrine activities, mast cells being the best studied, it has been shown that the granules are probably composed of a glycosaminoglycan matrix, the ion exchange properties of which retain both the amine and the often basic granule protein (3). Probably the neoplastic change of the cells impairs the storage capacity. Thus in mastocytosis the metachromatic granules are fewer, smaller and stain less intensely than usual (?). In all these cells we do not always know which one of the different substances accounts for the characteristic staining properties.

The varying frequency of granulated cells in MCT also corresponds well with the finding that immunofluorescence technique could only detect immunoreactive calcitonin in 40% of the cells in a case of MCT (4).

Even if only a minority of the MCT cells show the characteristic red granulation these are of great diagnostic importance (26). However it is of practical and theoretical interest to note that fine needle aspirates of other neuroendocrine and APUD-cell tumours also exhibit red granulated cells that are akin to those of MCT pheochromocytomas (Fig. 11) chemodectomas (Fig. 12) and carcinoid tumours. This resemblance may evoke differential diagnostic problems when the biopsy specimen is taken from a mediastinal or neck tumour. Sometimes mammary carcinomas and hypernephromas have red granulated cells though seldom or never creating a practical problem (own unpublished observations).

As the red granules of MCT are stained with May-Grünwald-Giemsa but not with hematoxylin-eosin we conclude that May-Grünwald-Giemsa should always be used as a routine staining technique for thyroid aspirates.

We also conclude from the present observations that the cloudy deposits seen in our MCT specimens consist of amyloid. As such deposits seem to lie present also within the cytoplasm of the tumour cells, it appears probable that this amyloid is produced within the tumour cell. This opinion is shared by other authors (11, 25). The nature of the amyloid of MCT has not been elucidated.

Amyloid was found in only 9/14 (64%) of our patients. However it is well known that the distribution of these deposits is uneven and patchy and some tumours contain only little amyloid. Thus it is not surprising that the yield of amyloid is rather haphazard in these aspirates. The fact that some biopsies were stained with alkaline Congo only after destaining of air-dried-fixed May-Grünwald-Giemsa smears may also have contributed negatively to the results.

The diagnostic results of fine needle aspiration biopsies as a whole may vary due to several factors. If the patient has a palpable abnormality and/or an abnormal scintiscan to guide the needle the chance of getting a representative cell yield is very good. The diagnosis should not be missed provided that the technical treatment of the smear is optimal. On the other hand to find the malignant MCT cells in patients with only small non-palpable tumour foci may certainly be difficult, e.g. as in the very early hereditary cases found in screening programs and in already surgically treated patients. However this method should be tried even in these cases as it

offers a chance to get a possible diagnosis with negligible discomfort and risk for the patient.

ACKNOWLEDGEMENT

This work was supported by grant from the Swedish Cancer Society (nos. 72:40 and 74:1).

REFERENCES

1. Almqvist, S., Tienhuis-Berg, M. & Wisked, B. Serum calcitonin in medullary thyroid carcinoma. Radioimmunoassay technique and diagnostic value. *Acta med. scand.* 196, 177, 1974.
2. Berg, B. Personal communication.
3. Bergqvist, U., Samuelsson, B. & Uvnäs, B. Chemical composition of basophil granules from isolated mast cells. *Acta physiol. scand.* 83, 76, 1971.
4. Bustolati, G., Foster, G. V., Clark, M. B. & Pearce, A. G. E. Immunofluorescent localization of calcitonin medullary (C cell) thyroid carcinoma, using antibody to the porcine hormone. *Vincennes Arch. Abt. Zellpath.* 2: 234, 1969.
5. Elinors, J. & Franzén, S. Thin-needle biopsies in the diagnosis of thyroid diseases. *Acta radiol.* 58, 3, 1965.
6. Franzén, S., Glertz, B. & Zapcek, J. Cytological diagnosis of prostatic tumours by transrectal sextant biopsy: a preliminary report. *Brit. J. Urol.* 1, 191, 1960.
7. Hazard, J. H., Hawk, W. A. & Cline, G. Medullary (solid) carcinoma of the thyroid—a clinicopathologic entity. *J. clin. Endocr.* 19, 152, 1959.
8. Ljungberg, O. On medullary carcinoma of the thyroid. *Acta path. microbiol. scand. sect. A Suppl.* 31, 1977.
9. Ljungberg, O. Cytologic diagnosis of medullary carcinoma of the thyroid gland. *Acta cytol.* 16: 253, 1972.
10. Ljungberg, O., Cederqvist, E. & von Stenutz, W. Medullary thyroid carcinoma and pheochromocytoma: A familial chromaffinomatosis. *Brit. med. J.* 1, 779, 1967.
11. Melvin, K. E., W. Miller, H. H. & Tishjian, A. R. Jr. Early diagnosis of medullary carcinoma of the thyroid gland by means of calcitonin assay. *New Engl. J. Med.* 285, 1115, 1971.
12. Nilsson, B. C-cells in non-malignant human goiters studied in fine-needle aspiration biopsy specimens. *Acta med. scand.* 191, 249, 1972.
13. Nilsson, O., Soderström, N. & Telander, M. Diagnosing thyroid carcinoma. *Lancet* 2: 74, 1970.
14. Pearce, A. G. E. Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C-cells and calcitonin. *Proc. roy. Soc. B* 170, 71, 1964.
15. — The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. *J. Histochem. Cytochem.* 17, 303, 1969.

16. Persson, P. S., Cytodagnosis of thyroiditis. *Acta med scand. Suppl.* 483 1968.
17. Pochter, H., Sweat, P. & Levine, M. On the binding of Congo red by amyloid. *J Histochem. Cytochem* 10: 355 1962.
18. Schinke, R. N. & Hartman, W. H. Familial amyloid-producing medullary thyroid carcinoma and pheochromocytoma. A distinct genetic entity. *Ann. Intern. Med.* 63 1027 1965.
19. Söderström, N. Fine-needle aspiration biopsy. Almqvist & Wiksell, Stockholm 1966.
20. Tateishi, R., Takahashi, Y. & Noguchi, A. Histologic and ultracytochemical studies on thyroid medullary carcinoma. *Cancer* 30: 735 1972.
21. Telenius, M. & Almqvist, S., Serum calcitonin as diagnostic tool in medullary carcinoma of the thyroid. *Acta endocr (Kbh)* Suppl 177 161 1973.
22. Tenkley-Berg, M., Almqvist, S. & Andersson, T. Screening for medullary carcinoma of the thyroid in families with Sipple's syndrome. To be published.
23. Tufvesson, G. & Tryding, N. Determination of diamine oxidase activity in normal human blood serum. *Scand. J. clin. Lab. Invest.* 24 163 1969.
24. Williams, E. B. Histogenesis of medullary carcinoma of the thyroid. *J. clin. Path.* 19: 114 1966.
25. Williams, E. D., Brown, C. L. & Dondach, I. Pathological and clinical findings in a series of 67 cases of medullary carcinoma of the thyroid. *J. clin. Path.* 19 103 1966.
26. Zajicek, J. Aspiration biopsy cytology part I. Cytology of supradaphragmatic organs, pp. 67-89. Karger, Basel 1974.
27. Åkerman, M. To be published.

THE THYROID IN ULCERATIVE COLITIS AND CROHN'S DISEASE

1 Thyroid Radiiodide Uptake and Urinary Iodine Excretion

Gunnar Järnerot

From the Department of Internal Medicine, Linköping University Medical School, Linköping, Sweden

Abstract. In order to investigate the prevalence of iodine depletion in chronic inflammatory bowel disease two separate studies have been performed. One was devoted to the 24-hour urinary iodine excretion and 30 patients with ulcerative colitis or Crohn's disease were examined and compared with 102 controls. In the other study the thyroid ^{125}I uptake was compared in 38 patients and 36 controls. Ten of the 30 patients with chronic inflammatory bowel disease had a 24-hour urinary iodine excretion less than 40 μg , compared with 5 of the 102 controls ($p < 0.01$). Sixteen of the 38 patients had a 24-hour thyroid ^{125}I uptake of 50% or more of the administered test dose, compared with 4 of the 36 controls ($p < 0.01$). These results are compatible with an increased occurrence of iodine deficiency in patients with chronic inflammatory bowel disease. Treatment with corticosteroids or Salazopyrin or milk-free diet did not influence these findings. No evidence was found of an impaired absorption of inorganic iodide from the gut.

Iodide is absorbed mainly from the small bowel (17). It then becomes part of the iodide pool from which it is taken up by the thyroid for production of thyroxine and triiodothyronine. The thyroid hormones are distributed to the tissues where they are deiodinated. Part of the liberated iodide is used by the thyroid again and part is excreted, the chief excretory routes being the urine and the faeces.

An earlier study raised the possibility of iodine deficiency in chronic inflammatory bowel disease (5). The aim of the present study was to compare the prevalence of iodine depletion in patients with chronic inflammatory bowel disease and healthy controls.

MATERIAL AND METHODS

Two separate studies were undertaken, in each of which the patients were asked to complete a questionnaire about their dietary habits with special regard to the consumption

of sea fish, milk and the use of iodized table salt and cooking. The laboratory analyses were made in the department of Clinical Chemistry, Linköping.

In the first study 4-hour urine samples were obtained from a group of patients with inflammatory gut disease (women 15, men 15 and 25 women with the mean age of 39 years (range 13-57)). They were analysed for iodine excretion in the Technicon PB1 AutoAnalyzer (Technicon Instrument Ltd) without pretreatment with an ascorbic acid reagent. Ten of the 30 patients had distal ulcerative colitis and 10 had ulcerative or universal disease. Twenty-eight of the patients had Crohn's disease, four of them had small anorectal lesions, 11 had more widespread colonic lesions and in 13 the small bowel was chiefly affected. In patients the colitis was reclassified.

Each patient had sigmoidoscopy and rectal biopsy performed together with radiologic examination of the colon and small bowel. Depending on the results of these studies and the clinical history diagnosis of ulcerative colitis (UC) or Crohn's disease was made. A differential diagnosis could not be made in 16 of the cases. During the time of the study 15 patients were treated with corticosteroids and 32 with Salazopyrin. Forty patients, of whom 15 were on milk-free diet, answered the dietary questionnaire. This group was compared with 102 controls (38 men, 64 women) without clinical evidence of thyroid or gastrointestinal disease. The mean age was 30.8 years (range 13-50).

In the second study the thyroid radiiodide uptake level was measured 24 hours after an oral test dose of $10 \mu\text{Ci } ^{125}\text{I}$, as potassium iodide. In separate groups of 20 males and 18 females with chronic inflammatory gut disease. The mean age of the patients was 29.4 years (range 16-57). The measurements were made according to the recommendations of the IAEA consultants group (14). In each case the urinary radioiodine excretion was determined from a 24-hour sample following the test dose. In 35 of these 38 patients the 4-hour urinary iodine excretion was also measured within a few days before or after the ^{125}I study. These 35 patients are included in the group of 30 patients in the first study. Five of the 38 patients had distal and more extensive or universal ulcerative colitis. Twenty-three patients had Crohn's disease, three of them had mainly anorectal changes, nine of the others had more widespread

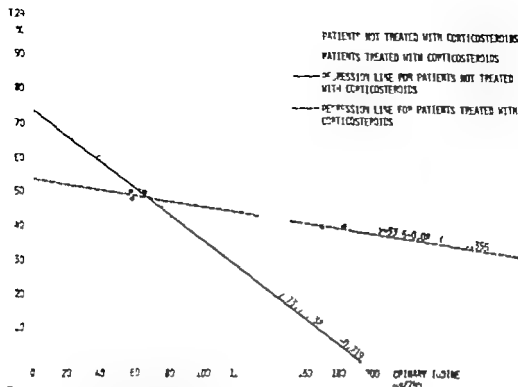


Fig 1 Correlation between the 4-hour thyroid ^{125}I uptake (T_{24}) and the 4-hour urinary iodine excretion in 35 patients with inflammatory bowel disease

our urinary iodine excretion of $40\text{ }\mu\text{g}$ or less is a reliable indication of iodine deficiency and an excretion of $40\text{--}70\text{ }\mu\text{g}$ is suggestive of incompletely filled iodine stores.

Corticosteroids diminish the thyroid accumulation of radioiodide and increase its urinary excretion (4, 9, 13). These effects are dose-dependent (1, 11). In the present study no significant differences were found between patients treated with corticosteroids and those who did not receive such treatment. However, if the correlation between the 4-hour thyroid ^{125}I uptake and the daily urinary iodine excretion is considered, it appears as if corticosteroids do influence these variables and one of them more than the other. As patients with more severe disease are more likely to be treated with corticosteroids, it is possible that they in fact had a more pronounced iodine depletion than the other patients.

Salicylate, which is a constituent of Salazopyrin[®], alters the protein binding of thyroxine (13, 22) and increases its turnover (2, 1). Hence it could have influenced the results. However, after ingestion of Salazopyrin[®] in therapeutic dosage, the serum concentrations of 5-aminosalicylic and acetyl-5-aminosalicylic acids are very low (16). In the present

study no significant differences were found between patients treated with Salazopyrin[®] and those who did not receive such treatment.

The dietary intake of iodine has a great influence on observations of the type made in the present study. The most important iodine sources are in foods such as sea fish, milk and iodized salt. In the present study the actual amounts of iodine ingested were not measured, but the consumption of sea fish was the same for patients and controls and all used iodized salt for cooking. Some of the patients were on a milk-free diet, but this did not significantly affect either their urinary iodine excretion or thyroid radioiodide uptake as compared with those patients who were on a non-restricted diet.

The mechanism of iodine depletion does not appear to depend on a reduced absorption of inorganic iodine from the inflamed gut, as the same proportion of the ^{125}I tracer dose could be detected in the urine and thyroid in patients and controls. Excessive loss of protein into the gut lumen is a well known complication of both UC and Crohn's disease. As thyroxine is protein-bound, it would be tempting to explain the increased prevalence of iodine depletion in these diseases as the result of a protein-losing enteropathy.

pathy. In fact a recent study has shown that thyroxine has an increased daily fractional turnover in UC and Crohn's disease (7). The thyroxine pool was very small in some patients. In some of the severely ill patients the daily disposal of thyroxine iodine was excessive and it is likely that, if the disease is of long duration this could be a cause of iodine depletion in patients with UC or Crohn's disease.

In chronic inflammatory bowel disease a certain amount of bleeding often occurs even in cases in which no blood has been observed macroscopically in the stools (18) which could contribute to the iodine losses. In that respect it is interesting to note that the male patients had results almost identical with those of the female controls of whom most were in the fertile ages and had regular menstruations.

The practical implications of these findings are not yet fully evaluated but it may be relevant that a recent study in Oxford (6) has shown a significantly higher frequency of simple goitre in patients with ulcerative colitis than in controls matched for age and sex.

REFERENCES

- Bernow, S. A. & Yalow, R. S. The effect of cortisone on the iodine accumulating function of the thyroid gland in euthyroid subjects. *J. clin. Endocr.* 12: 407 1952.
- Bricker, M. S. & Hlad, C. J. Jr Observations on the mechanism of the renal clearance of I^{131} . *J. clin. Invest.* 34 1057 1955.
- Ek, B., Johansson, S. & von Porat, B. Iodide repletion test in an endemic goitre area. Risk of iodine-induced hyperthyroidism. *Acta med. scand.* 173: 341 1963.
- Frederickson, D. S., Forsham, P. H. & Thorn, G. W. The effect of massive cortisone therapy on measurement of thyroid function. *J. clin. Endocr.* 12: 541 1952.
- Jarnerot, G. Thyrotoxicosis in ulcerative colitis? *Läkartidningen* 69: 570 1972.
- Jarnerot, G., Khan, A. K. A. & Tronlove, S. C. The thyroid in ulcerative colitis and Crohn's disease. II. Thyroid enlargement and hyperthyroidism in ulcerative colitis. *Acta med. scand.* 197: 83 1975.
- Jarnerot, G., Tronlove, S. C. & Warner, G. T. The thyroid in ulcerative colitis and Crohn's disease. III. The daily fractional turnover of thyroxine. *Acta med. scand.* 197: 89 1975.
- Keating, F. R. J. & Albert, A. The metabolism of iodine in man as disclosed with the use of radiiodine. *Recent Progr. Hormone Res.* 4: 429 1949.
- Kohl, F. R. Jr & Ziff, M. J. Alteration of thyroid function by ACTH and cortisone. *J. clin. Endocr.* 12: 554 1952.
- Kutschera-Alchberg, H. Jodmangel und Jodmangelkrankheiten. *Mitsch. med. Woch.* 103: 345 1961.
- Magalotti, M. F., Hummon, I. F. & Herschbiel, E. The effect of disease and drugs on the twenty-four hour I^{131} thyroid uptake. *Amer. J. Roentgenol.* 81: 47 1959.
- Myant, N. B., Corbett, B. D., Howser, A. J. & Pochin, E. E. Distribution of radiiodide in man. *Clin. Sci.* 9: 405 1950.
- Osorio, C. Effect of salicylate and dextrophenol on the binding of thyroid hormones by human and rat serum proteins at pH 7.4. *J. Physiol. (Lond.)* 163: 151 1962.
- Report of group of consultants of the International Atomic Energy Agency. The calibration and standardization of thyroid radiiodine uptake measurements. *Phys. & Med. Biol.* 6: 533 1961.
- Riggs, D. S. Quantitative aspects of iodine metabolism in man. *Pharmacol. Rev.* 4: 284 1952.
- Schröder, H. & Campbell, D. E. S. Absorption, metabolism and excretion of salicylazosulphapyridine in man. *Clin. Pharmacol. Ther.* 13: 539 1972.
- Small, M. D., Bezman, A., Longman, A. E., Fennel, A. & Zameck, N. Absorption of potassium iodide from the gastro-intestinal tract. *Proc. Soc. exp. Biol. (N.Y.)* 106: 450 1961.
- Stack, D. H. R., Smith, T., Hywell Jones, J. & Fletcher, J. Measurement of blood and iron loss in colitis with a whole-body counter. *Gut* 10: 769 1969.
- Stanbury, J. B., Brownell, G. L., Riggs, D. S., Perinetti, H., DeCastillo, E., Ito, J., Housay, A., Trucco, E. & Yacofano, A. C. The iodine deficient human thyroid gland. *J. clin. Endocr.* 1: 191 1952.
- Wayne, E., Koutras, D. A. & Alexander, W. H. Clinical aspects of iodine metabolism. Blackwell Scientific Publ. Oxford 1964.
- Woebber, K. A. & Ingber, S. H. The effects of noncalorigenic congeners of salicylate on the peripheral metabolism of thyroxine. *J. clin. Invest.* 43: 931 1964.
- Wolff, J., Standaert, M. H. & Rail, J. E. Thyroxine displacement from serum proteins and depression of serum protein-bound iodine by certain drugs. *J. clin. Invest.* 40: 1373 1961.
- Zegg, W. & Perry, W. F. The influence of adrenal and gonadal steroids on the uptake of iodine by the thyroid gland. *J. clin. Endocr.* 13: 712 1952.

Table I Brief details of the patients and controls with a history of hyperthyroidism

Case no	Present age (y)	Symptoms at the time of diagnosis	Involvement of colon	Time of occurrence of hyperthyroidism (y)	
				Before UC	After UC
Among 300 patients with ulcerative colitis					
1	46	Hyperthyroid symptoms. Operated on. Histology: thyrotoxicosis	Extensive colitis	16	
2	47	Tremor. Myopathy. Thyroid bruit. Thyroid tests and radiolodide uptake test within hyperthyroid range. Treated with antithyroid drugs	Universal colitis		10
3	50	Tremor. Sweating. Palpitations. Nervous. Eye symptoms. Thyroid radiolodide uptake test within hyperthyroid range. Treated with antithyroid drugs	Distal colitis	8	
4	57	Tremor. Sweating. Eye symptoms. Thyroid test and radiolodide uptake test within hyperthyroid range. Treated with radiolodide	Universal colitis	1	
5	58	Weight loss. Eye symptoms. Thyroid tests and radiolodide uptake test within hyperthyroid range. Treated with radiolodide	Distal colitis	3	
6	59	Hyperthyroid symptoms. Operated on. Histology: thyrotoxic goitre	Distal colitis		12
7	60	Weight loss. Unilateral exophthalmos. Thyroid tests within hyperthyroid range. Treated with antithyroid drugs	Universal colitis		19
8	66	Nervous. Exophthalmos. Operated on. Histology: thyrotoxicosis	Distal colitis	41	
9	70	Tremor. Nervous. Exophthalmos. Sweating. Radiolodide uptake test within hyperthyroid range	Universal colitis	12	
10	73	Weight loss. Eye symptoms. Myopathy. Thyroid test and radiolodide uptake test within hyperthyroid range. Treated with radiolodide and later antithyroid drugs after recurrence	Distal colitis	0*	0*
11	78	Hyperthyroid symptoms. Operated on. Histology: thyrotoxicosis. Later pernicious anaemia	Distal colitis	9	
# 600 controls					
1	38	Exophthalmos. Sweating. Weight loss. Nervous. Thyroid tests within hyperthyroid range. Treated with antithyroid drugs			
2	44	Exophthalmos. Weight loss. Thyroid tests within hyperthyroid range. Treated with antithyroid drugs			
3	47	Exophthalmos. Weight loss. Thyroid tests and radiolodide uptake test within hyperthyroid range. Treated with antithyroid drugs			
4	56	Sweating. Palpitations. Weight loss. Treated with antithyroid drugs. Radiolodide treatment after recurrence			
5	59	Exophthalmos. Nervous. Weight loss. Operated on. Hospital notes lost			

The diagnoses of thyrotoxicosis and UC were made at the same time

cision of myxoedema that could not be confirmed. On the contrary her thyroid activity was not suppressible with triiodothyronine and such a finding is very rare in the absence of hyperthyroidism or a nodular goitre (14) which she did not have. However as she never had had thyrotoxic symptoms clinically she was not considered to be a case of hyperthyroidism. The frequency of a firm history of hyperthyroidism in UC patients was 3.7% (Table II). No difference

was found between patients with distal colitis and those with more substantial involvement of the colon in the liability to have a history of hyperthyroidism.

Among the controls five women gave a history of hyperthyroidism and had been treated for it. The details of these women are given in Table I. The frequency was 0.8% (Table II) i.e. significantly lower than in the UC group.

No cases of hypothyroidism were found in the UC

Table II Frequency of hyperthyroidism in ulcerative colitis patients compared with the frequency in controls

	N	Hyperthyroidism		Significance of the difference
			%	
UC	300	11	3.7	$\chi^2=9.19$ $=1$ $p<0.01$
Controls	600	5	0.8	

group. In the control group two men and two women were being treated for this disease. The diagnosis in two of these patients could be definitely confirmed from the notes. No cases of thyroiditis or malignant thyroid disease were observed.

One UC patient and five controls had been operated on for non-toxic goitres. In evaluating the number of observed thyroid enlargements in these two groups these postoperative cases were regarded as examples of enlarged thyroids even if the thyroid was of normal size at the time of the study. Table III shows the number of visible or palpable goitres found by the two observers. As expected there were differences between the findings of the two observers, but they both found a significantly higher frequency of enlarged thyroids in the UC group than in the control group.

Enlarged thyroids were noted with the same frequency among patients with distal colitis as among those with more widespread disease of the colon (Table IV). Likewise the duration of UC did not have any influence on the liability to an enlarged thyroid gland (Table V).

DISCUSSION

A study of this kind has two possible sources of error. One is whether the control group is representative of the population. Patients admitted to the medical wards come from all parts of the Oxford area. They have varying occupations and represent all social classes. Their visitors would have the same characteristics and therefore constitute a random sample of the population in the Oxford area.

The other difficulty is the interindividual disagreement on the size of the thyroid in borderline cases. For the individual observer the only reference point is his own experience of what is normal or abnormal. Thus observations are strictly comparable only when made by one and the same observer.

As expected there were differences between the findings of the two observers. However the important point is that independently they found a significantly higher frequency of enlarged thyroids in the UC group than in the control group.

It is common knowledge that simple goitre is usually associated with iodine deficiency. The findings of a raised thyroid radioiodide uptake and reduced urinary iodine excretion in patients with ulcerative colitis are consistent with the occurrence of an increased prevalence of iodine depletion in this disease (13). However it is not known whether these abnormalities of iodine metabolism are long standing or simply occur for brief periods during and soon after acute attacks of this disease. If the chance of developing a simple goitre is increased by iodine loss through the inflamed colonic mucosa in UC one might expect that patients with extensive oral colitis would show a higher prevalence of simple goitre than patients with distal UC and likewise patients with a long history of UC would show a higher prevalence of goitre than patients with a short history. Neither of these expectations has been supported by the findings of the present study. The explanation for the increased prevalence of simple goitre in subjects with UC remains uncertain.

The frequency of hyperthyroidism in UC patients found in the present study is the highest reported so far. This may be because all the patients and control were interviewed specifically about thyroid problems whereas routine case histories may be defective regarding the past history of the patients.

It is worth noting that as shown in Table I a majority of the UC patients with a history of hyperthyroidism had suffered from the hyperthyroidism before they developed the colitis. In other words the time of occurrence does not

Table III Frequency of definite thyroid enlargement in ulcerative colitis patients and in controls

	N	Enlarged thyroids		Significance of the difference
			%	
Observer 1				$\chi^2=6.899$ $=1$ $p<0.01$
UC	300	26	8.7	
Controls	600	26	4.3	
Observer 2				$\chi^2=4.342$ $=1$ $p<0.05$
UC	300	19	6.3	
Controls	600	20	3.3	

Table IV Frequency of enlarged thyroid gland in ulcerative colitis in relation to the maximum extent of the colitis as judged radiologically

The extent of the disease could not be decided in 5 subjects. Two of them were found to have enlarged thyroids by observer 1 and one by observer 2

Involvement of colon	N	Enlarged thyroids		Significance of the difference
		n	%	
Observer 1				
Universal or extensive colitis	122	11	9.0	$\chi^2=0.216$ $n=1$ $p>0.05$ N.S.
Distal colitis	173	13	7.5	
Observer 2				
Universal or extensive colitis	122	7	5.7	$\chi^2=0.048$ $n=1$ $p>0.05$ N.S.
Distal colitis	173	11	6.4	

support the view that hyperthyroidism is a complication of the bowel disease

It is widely believed that immunological disturbances participate in the pathogenesis of hyperthyroidism (5, 19, 22). Hyperthyroidism is known to be associated with some other diseases thought to be immunologically induced such as *a* gravida (8, 18, 21), Addison's disease (2, 15) and pernicious anemia (20, 25, 28). A similar ship is also illustrated by the association of Addison's disease with pernicious anemia (1, 2). There is also a strong association between myxoedema and pernicious anemia (27, 28).

The thyroid gland and the stomach both derive

Table V Frequency of enlarged thyroid gland in ulcerative colitis patients in relation to duration of the colitis

Duration (y)	N	Enlarged thyroids		Significance of the difference
		n	%	
Observer 1				
<8	155	12	7.7	$\chi^2=0.346$ $n=1$ $p>0.05$ N.S.
>8	145	14	9.7	
Observer 2				
<8	155	6	3.9	$\chi^2=3.278$ $n=1$ $p>0.05$ N.S.
>8	145	13	9.0	

from the primitive foregut and both have the ability to trap iodide (10, 17). It is therefore not surprising to find a high incidence of thyroid antibodies in pernicious anemia (4) and conversely a high incidence of antigastric antibodies in hyperthyroidism (29). In Addison's disease there is also a great proportion of patients with thyroid antibodies (2).

The colon does not have the same embryological origin as the thyroid gland nor does it have the ability to concentrate iodide. Thyroid antibodies occur in UC with the same frequency as in the normal population (17, 26, 30). In one study antigastric antibodies were found more frequently in UC patients than in controls but the difference was not statistically significant (30) while in another study these antibodies occurred with a similar frequency in UC patients and controls (17). Adrenal antibodies do not occur with undue frequency in UC (17).

Consequently it is more difficult to explain an association between thyroid disease and ulcerative colitis on an immunological basis than the association between thyroid disease and pernicious anemia. The isolation of a thyroid-stimulating factor from *Clostridium perfringens* (16) may give a clue to the relationship between hyperthyroidism and chronic inflammatory bowel disease. However this factor needs to be further studied before any conclusions can be drawn. It is common clinical experience that both hyperthyroidism and acute attacks of UC often seem to be initiated by psychological trauma in the setting of emotionally stressful situations. It is therefore tempting to ascribe the association between these two conditions to psychosomatic factors which could initiate hyperthyroidism in some subjects, UC in others and both these diseases in a few. However McKenzie (19) has said of hyperthyroidism that "despite a vast literature on the subject there is no convincing evidence of how or if a psychological or emotional determinant is concerned in the apparently complex pathogenesis and the same is certainly true of ulcerative colitis".

ACKNOWLEDGEMENT

E. J. was the holder of fellowship in the European Science Exchange Programme and A. K. A. K. the holder of Commonwealth fellowship.

REFERENCES

- Berlin R. Addison's disease: Familial incidence and occurrence in association with pernicious anemia. *Acta med scand.* 144: 1, 1952.

4. Blizard, R. M. & Kyle, M. Studies of the adrenal antigens and antibodies in Addison disease. *J clin. Invest.* 42: 1653 1963.
5. Clubb, J. S., Black, P. J. & Wallace, D. C. An association of thyroid disease, ulcerative colitis and diabetes mellitus. Report of three cases in young women. *Aust. Ann. Med.* 19: 159 1970.
6. Doniach, D., Robt, I. M. & Taylor, K. B. Autoimmune phenomena in pernicious anaemia. Serological overlap with thyroiditis, thyrotoxicosis, and systemic lupus erythematosus. *Brit. med. J.* 1: 1374 1963.
7. Dorrington, K. J. & Munro, D. S. The long-acting thyroid stimulator. *Clin. Pharmacol. Ther.* 7: 788 1966.
8. Edwards, P. C. & Truelove, S. C. The course and prognosis of ulcerative colitis. Part III Complications. *Gut* 1: 1 1964.
9. Fredrickson, D. S. Effect of massive corticosteroid therapy on thyroid function. *J. clin. Endocr.* 11: 760 1951.
10. Garsen, L. H. & Levitan, S. Myasthenia gravis and thyroid function. *Arch. Neurol.* 18: 107 1968.
11. Gattassus, C. F., Myers, W. R., Arnold, J. W. & McCormack, W. M. Thyroid disorders in Addison disease. 2. Grave disease. *Mayo Clin. Proc.* 39: 939 1964.
12. Goldsmith, R. E., Stevens, C. D. & Schiff, L. Concentration of iodine in the human stomach and other tissues as determined with radioactive iodine. *J. Lab. clin. Med.* 35: 497 1950.
13. Golger, J. C., de Dombal, F. T., Watts, J. McK. & Wilkinson, G. Ulcerative colitis. Baillière Tindall and Co. London 1968.
14. Honour, A. J., Myant, N. B. & Rowlands, E. N. Secretion of radiolodine in digestive juices and milk in man. *Clin. Sci.* 11: 447 1952.
15. Jarnerot, O. The thyroid in ulcerative colitis and Crohn disease. I. Thyroid radiolodide uptake and urinary iodine excretion. *Acta med. scand.* 197: 77 1973.
16. Jarnerot, O. & Karlberg, B. E. A thyroid-suppression test, using a single large oral dose of thyroxine. *Läkartidningen* 70: 381 1973.
17. Klasson, C. H. L., van Dommelen, C. K. V. & van Unnik, J. A. M. Coincidence of adrenal atrophy haemolytic anaemias and hyperthyroidism, post followed by sudden death. *Acta med. scand.* 190: 78 1966.
18. Macchia, V., Bates, R. W. & Pastan, J. The purification and properties of thyroid-stimulating factor isolated from *Clostridium perfringens*. *J. biol. Chem.* 242: 3726 1967.
19. Marcusson, H. & Nerup, J. Fluorescent anti-thyroid and organ-specific antibodies in ulcerative colitis. *Scand. J. Gastroent.* 8: 9 1973.
20. McEachern, D. & Parnell, J. L. The relationship of hyperthyroidism to myasthenia gravis. *J. clin. Endocr.* 8: 842, 1948.
21. McKenzie, J. M. Humoral factors in the pathogenesis of Grave disease. *Physiol. Rev.* 48: 257 1968.
22. McNiel, G. P. Thyrotoxicosis associated with pernicious anaemia. *Amer. J. med. Sci.* 241: 336 1961.
23. Millikan, C. H. & Haines, S. F. The thyroid gland relation to neuromuscular disease. *Arch. intern. Med.* 92: 5 1953.
24. Ochi, Y. & DeGroot, L. J. Studies on the immunological properties of LATS. *Endocrinology* 83: 845 1968.
25. Powell, R. J., Shapiro, H. A. & Carbone, J. V. Therapeutic problems of ulcerative colitis with hyperthyroidism. *Amer. J. Gastroent.* 50: 116 1968.
26. Salner, J. W. Hypermotility of the gastro-intestinal tract in hyperthyroidism. *Amer. J. med. Sci.* 186: 73 1933.
27. Sternstein, T. Pernicious anaemia and Basedow disease. *Acta med. scand.* 104: 29 1940.
28. Thayer, W. R. J. & Spiro, H. M. Protein abnormalities in ulcerative colitis patients and their families. *Gastroenterology* 44: 444 1963.
29. Tudhope, O. R. & Wilson, O. M. Anaemia in hypothyroidism. *Quart. J. Med.* 29: 513 1960.
30. Watkinson, F. Megalocytic anaemias. *Lancet* 1: 336 1949.
31. Williams, M. J., Scott, G. B., Beck, J. S. & Blair, D. W. Antigastric antibodies in hyperthyroidism. Their relationship to impaired acid secretion. *Brit. med. J.* 1: 388, 1966.
32. Wright, R. & Truelove, S. C. Autoimmune reactions in ulcerative colitis. *Gut* 7: 22, 1966.

THE THYROID IN ULCERATIVE COLITIS AND CROHN'S DISEASE

III The Daily Fractional Turnover of Thyroxine

G Järnerot, S C Truelove and G T Warner

From the Nuffield Department of Clinical Medicine, the Radcliffe Infirmary, Oxford, ENGL

Abstract The daily fractional turnover of thyroxine (T_4) labelled with ^{125}I has been determined in 11 patients with ulcerative colitis (UC) or Crohn's disease and 8 controls. The daily fractional turnover of ^{125}I T_4 was significantly increased in the patient group. The daily total disposal of T_4 iodine was not significantly different although it was excessive in 3 of the 11 patients. The amount of T_4 in plasma did not differ significantly between the patients and the controls. It is concluded that the T_4 metabolism is disturbed in UC and Crohn's disease and that excessive losses of T_4 iodine can be a cause of iodine depletion in some patients with longstanding and severe disease.

An earlier study showed evidence suggestive of an increased prevalence of iodine deficiency in patients with ulcerative colitis (UC) or Crohn's disease (10). A hypothesis was put forward that the iodine depletion might be due to increased losses of thyroxine iodine. The present study was undertaken to find out if patients with UC or Crohn's disease had altered thyroxine (T_4) metabolism in keeping with this hypothesis.

MATERIAL AND METHODS

The experimental group consisted of five patients with UC and six with Crohn's disease. The mean age was 31.5 years (range 18-55). As shown in Table I four of the UC patients had extensive or universal disease and one had distal colitis. Of the patients with Crohn's disease three had extensive colonic disease, two widespread small bowel disease and one had ileocaecal inflammation. The diagnosis was based on the clinical history, the radiologic findings, the findings at sigmoidoscopy with rectal biopsy and, in six patients, on the findings at subsequent operation together with histologic examination of operation specimens.

Six of the 11 patients had lost considerable weight (3.3-12.7 mean 11.4 kg) and were judged clinically to be severely ill, while the remaining five were mildly or moder-

ately ill. At the time of the study seven (F.L. 1) were treated with oral or parenteral corticosteroids and three only for the latter half of the investigation. None had any clinical symptoms of dysfunction of the thyroid and none was receiving any drugs known to influence the thyroxine metabolism apart from corticosteroids. One patient (no. 10) had moderate fever at the time of the study while the others were afebrile. The patient group was compared with eight healthy controls, none of whom was on contraceptives or any other medication. The mean age was 35.9 years (range 27-47).

The thyroid was first blocked by giving 40 mg potassium iodide orally in two divided doses over the course of 4 hours and the blockade was maintained by 60 mg potassium iodide given orally twice daily throughout the study period. One day after commencing the thyroid blockade blood was drawn for estimation of T_4 in plasma. This was immediately followed by an i.v. injection of 10 μ Ci ^{125}I - T_4 in normal saline.

T_4 labelled with ^{125}I in 3, 5' position, with specific activity of 20-50 mCi/mg T_4 and a guaranteed content of ^{125}I as free iodide of less than 5% was obtained from the Radiochemical Centre, Amersham. On delivery it was diluted with 50% aqueous propylene-glycol in order to reduce the further liberation of free ^{125}I and sterilized by Millipore filtration. Each batch of ^{125}I - T_4 was used within a fortnight. Dr H. Johnston sterilized the ^{125}I - T_4 by ultrafiltration and Dr B. Klägdal, Linköping, Sweden, measured the T_4 in serum.

The study was performed using the whole-body counter described by Warner and Oliver (27). The value recorded 24 hours after the administration of ^{125}I - T_4 was considered as the 100% value, thereby allowing time for distribution of the ^{125}I T_4 in the body and also for excretion of ^{125}I -labelled impurities (chiefly free iodide) in the injected sample. Each subject was measured on the whole-body counter once daily for 6-10 days and the values were plotted semilogarithmically. The biological half-life ($T_{1/2}$) of ^{125}I - T_4 was calculated from the regression line. The fractional daily turnover of ^{125}I - T_4 was calculated from the relation $0.693/T_{1/2}$ (25). A thyroid count was performed 24 hours after the injection of ^{125}I T_4 and compared with a similar count of the thigh as control tissue. The distribution volume was calculated from the weight, using equation 5 as computed

Table II Results of the study and statistical calculations

ETI=extrathyroidal T iodine T_cI=T iodine

	B wt. (kg)	T _c -I/ (2.9-6.3 µg/ 100 ml)	Distribution volume (l)	T _{1/2} (d)	Fractional daily turnover (%)	ETI pool (µg)	ETI pool/ 70 kg (µg)	T _c -I dis- posal/d. (µg)	T _c -I dis- posal/d./70 kg (µg)
I. Mildly or moderately ill patients (n=5)									
Mean	61.7	3.7	10.7	6.14	11.4	389.7	447.3	44.9	51.0
S.D.	8.8	0.7	1.2	0.80	1.5	62.9	90.0	10.4	10
S.E.M.	3.9	0.3	0.5	0.36	0.7	28.1	40.2	4.6	4.6
II. Severely ill patients (n=6)									
Mean	51.2	3.8	8.8	4.20	17.0	316.8	478.0	51.9	79.0
S.D.	21.7	1.8	3.0	0.78	3.4	125.8	253.9	18.8	40.4
S.E.M.	8.8	0.7	1.2	0.32	1.4	51.4	103.6	7.7	16.5
III. All patient (n=11)									
Mean	46.0	3.6	9.7	5.09	14.5	350.0	464.1	48.7	66.3
S.D.	17.2	1.3	2.4	1.26	3.9	104.6	189.0	15.3	32.7
S.E.M.	5.2	0.4	0.7	0.38	1.2	31.5	57.0	4.6	9.9
IV. Controls (n=8)									
Mean	68.0	4.1	11.4	6.88	10.2	464.2	478.2	46.9	48.2
S.D.	6.7	1.0	0.7	0.69	1.0	135.8	113.1	13.1	10.7
S.E.M.	2.6	0.4	0.3	0.4	0.4	48.0	40.0	4.6	3.8
Significance of the differences									
I-II									
t	1.017	0.121	1.281	4.074	3.579	1.177	0.3	0.735	1.497
P	N.S.	N.S.	N.S.	<0.005	<0.01	N.S.	N.S.	N.S.	N.S.
III-IV									
t	1.772	0.886	1.839	3.627	3.039	1.958	0.178	0.279	1.496
P	N.S.	N.S.	N.S.	<0.005	<0.01	N.S.	N.S.	N.S.	N.S.
II-IV									
t	1.910	0.367	2.191	6.812	5.421	1.920	0.002	0.543	1.909
P	N.S.	N.S.	<0.05	<0.001	<0.001	N.S.	N.S.	N.S.	N.S.

DISCUSSION

The sensitivity of the whole-body counter used in this study is not dependent on the distribution of the isotope in the body (27). Hence, there is no need to measure the patient at different positions as was done by Oddie *et al.* (15). It is known that commercial ¹²⁵I-T contains radioactive impurities, mainly as iodide (26). A certain time is also required after injection for distribution and equilibration of the labelled compound. By using the value 3 hours after injection of ¹²⁵I-T as the 100% value the influence of radioactive impurities is largely eliminated and the time required for distribution and establishing a state of equilibrium is available. The T_{1/2} values and the daily fractional turnover in the controls are in good agreement with earlier published results as found by the blood disappearance curve (4, 6, 8, 14, 25) and also by whole-body counting (15).

Normally about 9-16% of the injected ¹²⁵I-T is excreted in the faeces within 3-11 days (1, 4, 8, 13, 15). Hence the difference in the frequency of bowel motions between the controls and the patients may influence the results, but it is probably of minor importance when the subjects are studied over a period of 6-10 days. This view is supported by the fact that seven of the patients, five of whom had considerably shortened T_{1/2}, had only one or two bowel motions daily so it seems unlikely that diarrhoea as such is the reason for the differences between the patients and the controls.

No appreciable differences were found in the present study between the patients who were treated with corticosteroids and those who were not. Neither did corticosteroids influence the prevalence of iodine depletion in an earlier study of patients with UC or Crohn's disease (10). T metabolism is said to

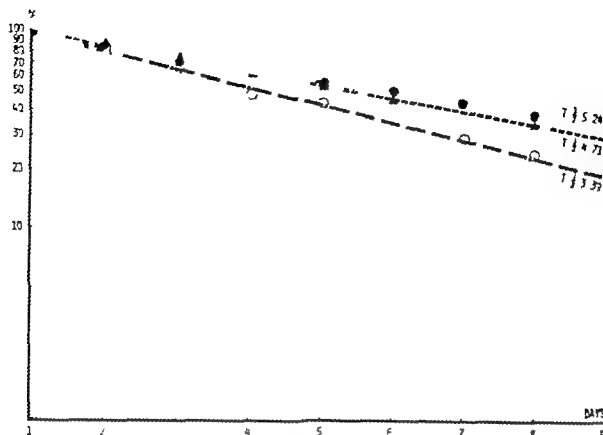


Fig 1 ^{125}I thyroxine disappearance curves in three of the patients.

be influenced by corticosteroids, although the results are rather inconsistent. Blomstedt and Einhorn (8) found a significant increase in urinary ^{125}I thyroxine after administration of fairly large doses of corticosteroids but they also noted a decreased faecal output, even if not of significant magnitude. Ingbar and Freinkel (9) found that corticosteroids induced an increased fractional turnover of T and a reduction of its distribution space with an unchanged amount of T iodine disposed of per day. Another study on the other hand showed a reduced fractional turnover rate of ^{125}I T and a fairly unchanged distribution volume (11). The daily T iodine disposal was reduced in most of the subjects studied. It therefore seems most unlikely that the short $T_{1/2}$ and increased daily fractional turnover found in the patients in the present study were due to treatment with corticosteroids.

It is well known that many major diseases change the $T_{1/2}$ of T (2, 7, 12, 20, 25) but exactly how the T turnover is regulated is not known. Both UC and Crohn's disease may cause heavy loss of protein through the inflamed gut (28). As T which is

protein-bound in plasma, moves across the capillary walls in the same way as albumin (17, 4) it is possible that increased amounts of T could be exuded into the gut lumen and there either be excreted in the faeces or be broken down with reabsorption of the iodide which is then partially excreted into the urine. It may be relevant that in the nephrotic syndrome considerable amounts of both T and T-binding globulin are found in the urine (21, 22). A thorough study of the nephrotic syndrome showed that patients with this condition had a small extra-thyroidal T iodine pool and a high daily fractional turnover of T (20). The daily disposal of T iodine was about the same as in normal controls but as the amount disposed of in the urine was so large the proportion metabolized by the body was less than normal. It was concluded that the increased daily fractional turnover was directly due to the increased losses of T in the urine and faeces. In the present study the daily disposal of T iodine was the same in the patients and the controls. If a protein-losing enteropathy and blood loss contribute to the losses perhaps a considerable proportion of the T iodine

disposed of per day could be lost in this way. It is also possible that a state of iodine depletion could be induced in some patients if the losses were large and the disease of long duration, and even more so if the iodine stores were small at the beginning of the illness. A raised radioiodide uptake which is found in states of iodine depletion was found in about one third of cases of nephrotic syndrome (21) and in 42% of patients with UC or Crohn's disease (10).

When the daily T₄ iodine disposal in the present study was corrected for weight, three of the 11 patients disposed of more than the mean ± 2.5 D of the control group. If such a process continues for long, a state of iodine depletion can be expected. This may explain the finding that 20% of a group of patients with chronic inflammatory bowel disease had a daily urinary iodine excretion of less than 40 μ g (10). In that study it was suggested that the increased prevalence of iodine depletion in UC and Crohn's disease could be due to excessive losses of T₄ and the present study gives support to that concept.

Another possible explanation of the short T_{1/2} of T₄ in UC and Crohn's disease could be an increased demand for triiodothyronine (T₃). It is well known that T₃ is partly the source of the body T₄ (5-19). Of the daily T₄ production 33% has been calculated to be converted into T₃ and this process accounts for 41% of the daily T₄ production (18). However preliminary results of a study in progress by the present authors on T₃ metabolism in UC and Crohn's disease have shown the same T₄ turnover rate in patients and controls and at least in some patients a low concentration of T₃ in serum. Therefore the increased T₄ turnover in these patients does not seem to be due to an increased conversion of T₄ into T₃.

The present study has shown that T₄ has an increased daily fractional turnover in severely ill patients with UC or Crohn's disease but further work is required to determine the metabolic pathways and routes of excretion.

ACKNOWLEDGEMENT

O. J. was the holder of a fellowship in the European Science Exchange Programme.

REFERENCES

- Albert, A. & Keating, F. R., Jr. Metabolic studies with ¹²⁵I labeled thyroid compounds. *J. clin. Endocr.* 9: 1406, 1949.
- Bellabarba, B., Inada, M., Varnano-Aharon, N. & Sterling, K. Thyroxine transport and turnover: major nonthyroidal illness. *J. clin. Endocr.* 28: 1023, 1968.
- Bloomstedt, B. & Enborn, J. Effect of cortisone on the peripheral degradation of ¹³¹I-thyroxine. *J. clin. Endocr.* 21: 181, 1965.
- Bloomstedt, B. & Plantin, L. O. The extrathyroidal distribution of ¹³¹I thyroxine. *Acta endocr. (kbb)* 43: 536, 1965.
- Braverman, L. E., Ingbar, S. H. & Sterling, K. Conversion of thyroxine to triiodothyronine in athyretic human subjects. *J. clin. Invest.* 49: 855, 1970.
- Cavallieri, R. R., Searle, O. L., Castle, J. N. & Dick, R. The kinetics of distribution between plasma and liver of ¹²⁵I-labeled L-thyroxine in man. Observations of subjects with normal and decreased serum thyroxine-binding globulin. *J. clin. Invest.* 45: 979, 1966.
- Gregersen, R. L. & Solomon, N. Acceleration of thyroxine and triiodothyronine turnover during bacterial pulmonary infections and fever: Implications for the functional state of the thyroid during stress and illness. *J. clin. Endocr.* 27: 93, 1967.
- Ingbar, S. H. & Freinkel, N. Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis. *J. clin. Invest.* 34: 808, 1955.
- The influence of ACTH, cortisone and hydrocortisone on the distribution and peripheral metabolism of thyroxine. *J. clin. Invest.* 34: 1375, 1955.
- Morner, G. The thyroid in ulcerative colitis and Crohn's disease. I. Thyroid radioiodide uptake and urinary iodine excretion. *Acta med. scand.* 197: 77, 1975.
- Kumar, R. S., Maza, B. V., Appleton, W. G. & Dowling, J. T. Effect of prednisone on thyroxine distribution. *J. clin. Endocr.* 28: 1335, 1968.
- Lutz, H. J., Gregersen, R. I., Spindling, S. W., Horvack, R. B. & Dawkins, A. T. J. Thyroxine binding proteins, free thyroxine and thyroxine turnover interrelationships during acute infection. *Ann. N.Y. Acad. Sci.* 130: 100, 1972.
- Myant, N. B. & Pochin, E. E. The metabolism of radiothyroxine in man. *Ch. Sci.* 9: 421, 1950.
- Nicoloff, J. T. & Dowling, J. T. Estimation of thyroxine distribution in man. *J. clin. Invest.* 47: 96, 1968.
- Odell, T. H., Fisher, D. A. & Rogers, C. Whole-body counting of ¹³¹I-labeled thyroxine. *J. clin. Endocr.* 24: 628, 1964.
- Odell, T. H., Meade, J. H. J. & Fisher, D. A. An analysis of published data on thyroxine turnover in human subjects. *J. clin. Endocr.* 26: 425, 1966.
- Oppenheimer, J. H., Bernstein, G. & Hase, J. Estimation of rapidly exchangeable cellular thyroxine from the plasma disappearance curves of simultaneously administered thyroxine-¹²⁵I and albumin-¹²⁵I. *J. clin. Endocr.* 46: 962, 1977.
- Pittman, C. S., Chamber, J. B. J. & Read, V. H. The extrathyroidal conversion rate of thyroxine to triiodothyronine in normal man. *J. clin. Invest.* 50: 1187, 1971.

Table 1 Results and statistical significance of the differences (S.E.M. within parentheses)

	TBG (15-35 µg/100 ml)	TBPA (140-220 µg/100 ml)	Albumin (42-51 g/l)	T ₄ (1.9-6.3 µg/100 ml)	T ₄ -test (80-120 °C)	Free T ₄ index (1-6.2 µg/100 ml)
A All patients (n=20)	25.0 (1.6)	84.3 (8.8)	36.3 (1.9)	4.4 (0.3)	106.0 (5.4)	4.4 (0.3)
B Corticosteroid-treated patients (n=10)	21.9 (1.5)	77.7 (14.0)	32.3 (2.3)	3.8 (0.5)	118.0 (7.6)	4.4 (0.5)
C Patients not treated with corticosteroids (n=10)	28.1 (2.5)	90.9 (11.2)	40.3 (2.6)	4.9 (0.5)	94.0 (5.8)	4.4 (0.2)
D Controls (n=20)	19.3 (0.9)	142.8 (7.9)	46.4 (1.4)	4.4 (0.2)	93.8 (3.7)	3.9 (0.2)
Statistical significance of the differences						
A-D	t=3.15 p<0.005	t=4.92 p<0.001	t=4.26 p<0.001	t=0.53 N.S.	t=1.85 N.S.	t=1.76 N.S.
B-D	t=1.54 N.S.	t=4.05 p<0.001	t=5.26 p<0.001	t=0.73 N.S.	t=3.23 p<0.01	t=1.01 N.S.
C-D	t=3.31 p<0.005	t=3.79 p<0.001	t=2.07 p<0.05	t=1.79 N.S.	t=0.03 N.S.	t=2.12 p<0.05
B-C	t=2.14 p<0.05	t=0.73 N.S.	t=2.32 p<0.05	t=1.35 N.S.	t=2.50 p<0.05	t=0.09 N.S.

Both the corticosteroid-treated patients and those who did not receive such treatment had significantly lower TBPA and albumin values in serum than the controls. On the other hand patients receiving such treatment had significantly lower albumin values than patients not receiving such treatment. TBPA values did not differ significantly in this respect. The correlation between TBPA and albumin was significant when estimated for patients and controls as one group ($r=0.620$, $p<0.001$) but insignificant in the patient group alone ($r=0.429$, N.S.) indicating that the two proteins do not behave identically.

The T_4 -test values were higher in the patients than in the controls but the difference was not significant. Treatment with corticosteroids resulted in significantly higher T_4 -test values compared to the controls and also to the patients not receiving such treatment.

The free T_4 index was similar in the patient and the control groups. Patients who did not receive corticosteroid treatment had significantly higher free T_4 index than the controls. However in the total patient group only one patient with a free T_4 index of 8.7 had a value above the upper normal limit.

The TSH level was normal in all patients (mean

4.9 range <3-10 µU/ml) and in all controls (mean 6.2 range <3-10 µU/ml).

DISCUSSION

Thyroxine in serum is normally bound by TBG (75%), TBPA (15%) and albumin (10%) (28). Hypoalbuminemia is a well known consequence of UC and Crohn's disease due to increased faecal losses of albumin (27). This was probably the cause of the low serum albumin in the present patients.

TBPA is usually decreased in systemic diseases (1, 11, 15, 17, 22). The decreased TBPA concentration has been attributed to decreased synthesis (15, 25) but also in some cases to increased degradation (18). As protein-bound T_4 moves across the capillary walls in the same way as albumin (16, 24) it is possible that increased amounts of TBPA could be exuded into the gut lumen and lost in the faeces. If so both decreased synthesis and increased degradation could be the cause of the reduced concentration of TBPA in UC and Crohn's disease.

The reason for the increase in TBG is not known as it is usually reported to be normal or reduced in systemic diseases with the exception of liver dis-

case and acute intermittent porphyria. In which raised TBG values have been noted (6-12). Liver disease is not infrequent in patients with UC or Crohn's disease. However usually the histological liver changes are mild and the liver is grossly diseased only in a small proportion of the patients (20-21). The present patients were not thoroughly investigated for the occurrence of liver disease but none of them had any clinical evidence of severe liver disease and bilirubin, transaminases and alkaline phosphatase in serum were normal.

Raised TBG and TBPA values have also been reported in a patient with anaalbuminemia with reversion to normal when the serum albumin concentration was restored (5). In the present study three patients with high TBG values (37-40 $\mu\text{g}/100\text{ ml}$ plasma) had normal albumin values indicating that the increase in TBG is not a compensatory phenomenon in UC and Crohn's disease.

Increased TBG concentrations have been reported in such geographical areas where iodine deficiency is common (26). Evidence of iodine deficiency is fairly common in UC and Crohn's disease (8), but our patients were not investigated for the occurrence of iodine deficiency.

No definite explanation of the increased concentration of TBG in UC and Crohn's disease can be given at present, but subclinical liver disease interfering with estrogen metabolism cannot be ruled out. This may be supported by the fact that the highest TBG values were found in female patients. On the other hand iodine deficiency which is more common in women, cannot be ruled out either.

The increase in TBG appears to compensate for the decrease in binding sites for T on TBPA and albumin as the T concentration in serum for both the patients and the controls was almost identical. T_4 , which otherwise would be bound to TBPA or albumin, is now probably bound to TBG. This would imply that a relatively greater proportion of the T binding sites on TBG are occupied than normally. The values from the T_3 -test, which reflects the number of free binding sites on TBG were also slightly although not significantly higher in the patients than in the controls. This would also help to preserve a normal concentration of free T in the serum, and the normal TSH values indicate that the concentration of free T is at least not subnormal. On the contrary the free T concentration in serum may be slightly higher in patients with UC or Crohn's disease who are not treated with corticosteroids

than in healthy controls if the free T index truly reflects the free T concentration in serum.

Corticosteroids are known to increase TBPA and decrease TBG (10-19). This is partly confirmed in the present study as corticosteroid-treated patients had significantly lower TBG values than patients who did not receive such treatment. On the other hand the controls and the corticosteroid-treated patients had the same TBG levels, indicating that corticosteroid treatment in UC and Crohn's disease induces a reduction of an otherwise increased TBG synthesis so that the TBG level becomes normal. This phenomenon together with the decreased concentrations of TBPA and serum albumin is probably the explanation of the slightly decreased T values in corticosteroid-treated patients compared to those not receiving such treatment. That corticosteroids reduce the T and increase the T_3 -test values is well known (2).

Patients treated with corticosteroids had a significantly lower serum albumin than the patients who did not have such treatment, while the TBPA values were lower but not significantly so. As corticosteroids increase the TBPA one would have expected these values to be higher in the corticosteroid-treated group. However there is reason to believe that the corticosteroid-treated patients were more severely ill than the patients who did not receive such treatment. As larger amounts of proteins are lost via the faeces in patients with severe inflammatory bowel disease than in patients with moderate or mild severity of the disease this could be the reason why the corticosteroid-treated patients had lower TBPA and albumin in serum than the patients not receiving such treatment. It is also possible that this could contribute to the lower TBG values in corticosteroid-treated patients compared to those who did not have such treatment.

The findings presented here show that in UC and Crohn's disease changes in the concentration of the thyroid hormone binding proteins can occur which sometimes may cause alterations in the thyroid tests so that hyperthyroidism can be suspected. In a patient with diarrhoea, weight loss and sometimes anxiety which all are common findings in UC and Crohn's disease a raised T and T_3 -test value make a misdiagnosis of hyperthyroidism easy. As a thyroid radioiodide uptake test often shows uptake values within the hyperthyroid range probably due to iodine deficiency (such a misdiagnosis) even to make (8). This condition has

"pseudo-hyperthyroidism" (7) and a further example of this condition is provided by one of the patients in the present series with a T₁ level of 7.0 µg/100 ml and a T₄-test value of 124%. His free T index of 8.7 was also high. After treatment of his bowel disease the thyroid tests became normal.

There is an increased prevalence of hyperthyroidism in patients with UC (9). Hence in patients with UC or Crohn's disease in whom the thyroid tests give rise to a suspicion of hyperthyroidism, which is not clinically clear-cut, it is necessary to perform a triiodothyronine suppression test in order to confirm or rule out a diagnosis of hyperthyroidism.

REFERENCES

1. Bellabarba, D., Imada, M., Vanzano-Aharon, N. & Sterling, K.: Thyroxine transport and turnover in major nonthyroidal illness. *J. clin. Endocr.* 28: 1023 1968.
2. Blomstedt, B. & Einhorn, J.: Effect of cortisone on the FBI and the resin uptake of ¹²⁵I-triiodothyronine. *Metabolism* 16: 319 1967.
3. DiGiubo, W., Michalski, Z., Weinhold, P. A., Hamilton, J. R. & Thoma, G. J.: Use of agar gel electrophoresis and autoradiography to measure thyroxine-binding protein capacities. *J. Lab. clin. Med.* 64: 349 1964.
4. Doonan, B. T., Watson, W. A. & Biggs, H. G.: Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. chim. Acta* 31: 87 1971.
5. Hollander, C. S., Bernstein, G. & Oppenheimer, J. H.: Abnormalities of thyroxine binding in analbuminemia. *J. clin. Endocr.* 28: 1064 1968.
6. Hollander, C. S., Scott, R. L., Tachody, D. P., Perfroth, M., Waxman, A. & Sterling, M.: Increased protein-bound iodine and thyroxine-binding globulin in acute intermittent porphyria. *New Engl. J. Med.* 277: 995 1965.
7. Järnerot, G.: Thyrotoxicosis in ulcerative colitis? *Läkartidningen* 69: 570 1972.
8. —: The thyroid in ulcerative colitis and Crohn's disease. I: Thyroid radioiodide uptake and urinary iodine excretion. *Acta med. scand.* 197: 77 1975.
9. Järnerot, G., Khan, A. K. A. & Truelove, S. C.: The thyroid in ulcerative colitis and Crohn's disease. II: Thyroid enlargement and hyperthyroidism in ulcerative colitis. *Acta med. scand.* 197: 83 1975.
10. Kumar, R. S., Musa, B. V., Appleton, W. B. & Dowling, J. T.: Effect of prednisone on thyroxine distribution. *J. clin. Endocr.* 28: 1335 1968.
11. Lutz, H. J., Gregerman, R. I., Spaulding, S. W., Hornick, R. B. & Dawkins Jr. A. T.: Thyroxine binding proteins, free thyroxine and thyroxine turnover interrelationships during acute infectious illness in man. *J. clin. Endocr.* 35: 230 1972.
12. McCosson, J., Row, V. V. & Volpé, R.: The influence of liver damage in man on the distribution and disposal rates of thyroxine and triiodothyronine. *J. clin. Endocr.* 34: 144 1972.
13. Norrila, H.: A simplified technique for the triiodothyronine test (T₃-test) with Sephadex. *Scand. J. clin. Lab. Invest. Suppl.* 86: 177 1965.
14. Odell, W. D., Rayford, P. L. & Ross, G. T.: Simplified partially automated method for radioimmunoassay of human thyroid stimulating growth, luteinizing, and follicle stimulating hormones. *J. Lab. clin. Med.* 70: 973 1967.
15. Oppenheimer, J. H.: Role of plasma proteins in the binding, distribution and metabolism of the thyroid hormones. *New Engl. J. Med.* 278: 1153 1968.
16. Oppenheimer, J. H., Bernstein, G. & Hasen, J.: Estimation of rapidly exchangeable cellular thyroxine from the plasma disappearance curves of simultaneously administered thyroxine-¹²⁵I and albumin-¹²⁵I. *J. clin. Endocr.* 46: 762 1967.
17. Oppenheimer, J. H., Squeer, R., Surks, M. I. & Hauer, H.: Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in nonthyroidal illness. *J. clin. Invest.* 42: 1769 1963.
18. Oppenheimer, J. H., Surks, M. I., Bernstein, G. & Smith, J. C.: Metabolism of iodine 131-labeled thyroxine-binding prealbumin in man. *Science* 149: 748 1965.
19. Oppenheimer, J. H. & Werner, S. C.: Effect of prednisone on thyroxine binding proteins. *J. clin. Endocr.* 26: 715 1966.
20. Perrett, A. D., Higgins, G., Johnston, H. H., Massarelli, G. R., Truelove, S. C. & Wright, R.: The liver in Crohn's disease. *Quart. J. Med.* 40: 187 1971.
21. —: The liver in ulcerative colitis. *Quart. J. Med.* 40: 211 1971.
22. Richards, J. H., Dowling, J. T. & Ingbar, S. H.: Alterations in the plasma transport of thyroxine in sick patients and their relation to the abnormality in Grave's disease. *J. clin. Invest.* 38: 1035 1959.
23. Seligson, H. & Seligson, D.: Measurement of thyroxine by competitive protein binding. *Clin. chim. Acta* 38: 199 1972.
24. Simpson-Morgan, M. W. & Irvine, C. H.: Transcapillary exchange of protein-bound hormones from the bloodstream into the tissue fluid. *Acta endocr. (Kbh.) Suppl.* 158: 128 1972.
25. Socolow, E. L., Woebber, K. A., Purdy, R. H., Holloway, M. T. & Ingbar, S. H.: Preparation of ¹²⁵I-labeled human serum prealbumin and its metabolism in normal and sick patients. *J. clin. Invest.* 44: 1600 1965.
26. Wellby, M. L., Pharoah, P. D. & Hetzel, B. S.: Changes in serum thyroxine-binding proteins in endemic goitre and endemic cretinism. *Clin. Endocr.* 3: 49 1974.
27. Wetterfors, J., Ljundahl, S.-O., Plantin, L. O. & Berke, G.: Hypoalbuminemia in ulcerative colitis and certain forms of enteritis. *Acta med. scand.* 174: 529 1963.
28. Woebber, K. A. & Ingbar, S. H.: The contribution of thyroxine-binding prealbumin to the binding of thyroxine in human serum as assessed by immunoadsorption. *J. clin. Invest.* 47: 1710 1968.

INHIBITION OF ADRENAL FUNCTION IN MAN BY HEPARIN OR HEPARINOID Ro 1-8307

P W C Kloppenborg, A F Casparie Th J Benraad and C. L. H. Majoor

*From the Division of Endocrinology the Department of Medicine University Hospital St. Radboud
Nijmegen The Netherlands*

Abstract Heparin and the heparinoid Ro 1-8307 inhibited the secretory rate of aldosterone in physiological or pathological aldosteronism to the level found in normal subjects on liberal sodium intake. In addition, these compounds inhibited corticosterone biosynthesis, although less markedly than that of aldosterone. Indications of interference with cortisol production have not been found. During drug treatment angiotensin, in doses of 5-10 mg/kg b.wt./day, did not stimulate aldosterone secretion. ACTH responsiveness of the adrenals—indicated by the fractional increases of both aldosterone and corticosterone secretory rates—remained unchanged. In two studies heparin had no consistent effect on plasma renin activity.

Heparin and related polysulphated polysaccharides like Ro 1-8307 (N-formyl-chitosan polysulphuric acid) have been shown to produce natriuresis and potassium retention (300-400 mg of regular heparin being equivalent to 600-800 mg of Ro 1-8307) (24, 25, 27, 31, 32). These effects proved to be caused by a decrease of the excretory and secretory rate of aldosterone both in normal subjects on low sodium intake and in patients with secondary (5, 13, 17, 22, 23, 31, 37) and primary aldosteronism (1, 15, 16, 19).

The mechanism by which these substances act on adrenal steroidogenesis *in vivo* is by no means clear. Decreased secretion of 18-hydroxycorticosterone has been observed by Abbott et al (1) to occur simultaneously with decreased aldosterone secretion during treatment of hypertensive subjects with Ro 1-8307. Therefore these authors concluded that the site of action of this heparinoid in adrenal steroidogenesis is prior to the synthesis of 18-hydroxycorticosterone. Coen et al (15) observed decreased excretion of an 18-hydroxycorticosterone metabolite 18-hydroxytetrahydro-11-dehydrocorticosterone during administration of Ro 1-8307 to

three patients with primary aldosteronism simultaneously the excretory rate of a corticosterone metabolite tetrahydrocorticosterone was reported to remain unchanged. These investigators speculated that the heparinoid might block the conversion of corticosterone to 18-hydroxycorticosterone. As a consequence they reasoned that normal subjects treated with the heparinoid, should have a higher rate of corticosterone production than controls. This would occur under these conditions because of increased activity of the aldosterone biosynthetic pathway due to the sodium loss caused by the treatment with the heparinoid.

In this study observations are reported of the effect of heparin and Ro 1-8307 on the secretory rates of aldosterone and corticosterone, the plasma renin activity (PRA) and the adrenal responsiveness to ACTH and angiotensin.

MATERIAL AND METHODS

Clinical

Fourteen normal subjects or patients without discernible disturbances of the renin-angiotensin-aldosterone system, five normal subjects or patients with secondary aldosteronism due to antihypertensive treatment and two patients with deep femoral vein thrombosis treated with the conventional anticoagulant, acenocoumarol (Sintrom, Geigy Basle, Switzerland) and diuretics participated in this study.

In all the studies the patients maintained a constant diet containing about 15 mmol sodium. Urine collection was checked daily for completeness by determination of creatinine content, and the excretion of sodium and potassium was measured. After equilibration aldosterone and corticosterone secretory rates and the influence of heparin or Ro 1-8307 (prepared and provided by Hoffman-La Roche Laboratories, Basle, Switzerland) on these secretory rates were determined. Heparin (ThromboSqualin Organon Osa, The Netherlands) (200-400 mg daily) was given i. m. and the heparinoid Ro 1-8307 (400-800 mg daily) i. m. or i. v. both in divided doses. In number of

Present address: Department of Medicine Hospital De Weezenlanden Zwolle The Netherlands.

studies the effects of angiotensin or ACTH were determined. These polypeptides were given by continuous I. infusion over a 4-hour period angiotensin (Hypertensin Ciba, Basle Switzerland) in doses of 5–10 ng/min/kg b wt. ACTH (ACTH-Organon, Oss, The Netherlands) in a total dose of 120 U.

In two studies aldosterone secretory rates and PRA were measured repeatedly on alternating days before, during and after heparin treatment. All aldosterone and corticosterone secretory rates were measured in patients during 24 hours of recumbency. PRA's were measured in blood taken at noon after 3 hours of quiet ambulation or sitting in chair.

Laboratory procedures

Aldosterone secretory rates were determined by an earlier described double isotope dilution derivative technique (6, 7). In 40 normal subjects on a dietary sodium intake of about 115 mmol a mean value of 135 ± 49 (1 S.D.) $\mu\text{g}/24$ h was found.

Corticosterone secretory rates were also measured by double isotope dilution derivative method (11). This method differs from Peterson and Pierce's (9) by the introduction of thin layer chromatography. Four μCi of $1,2\text{-}^3\text{H}$ -corticosterone (New England Nuclear Corporation) is injected I. After treating an aliquot of the 24-hour urine with β -glucuronidase the dichloromethane extract is chromatographed on a silica gel layer twice in the system chloroform-ethanol, 99:1 (v/v) and once in the system chloroform-methanol-water 94:6:0.5 (v/v). The area which contains the radio-labeled corticosterone metabolites, tetrahydro- and alicetetrahydrocorticosterone is eluted and chromatographed on a second silica gel layer in the system chloroform-acetone-water 60:4:0.5 (v/v). Separation of the two metabolites is achieved by paper in the system cyclohexane-benzene-water 100:100:100:90 (v/v). One metabolite acetylated with ^3H -acetic anhydride (0.2 mCi/mmol, diacetate) formed is purified by thin layer chromatography in the system chloroform-acetone 98:2 (v/v), subsequently by paper chromatography in the system cyclohexane-benzene-methanol-water 100:25:100:10 (v/v) and finally by thin layer chromatography in the system chloroform-acetone, 95:5 (v/v). Purity of the acetate I. assessed by comparing the $^3\text{H}/^3\text{H}$ ratios after each of the last two chromatographic steps. In a number of determinations the method was checked by comparing the $^3\text{H}/^3\text{H}$ ratios of the diacetates of both metabolites and by hydrolysis of the diacetates of one metabolite into its monacetate. By the last procedure the $^3\text{H}/^3\text{H}$ ratio was increased by hydrolysis was 2.02 ± 0.09 (1 S.D.). In 16 normal subjects a mean secretion rate of 3.8 ± 1.0 $\mu\text{g}/24$ h was found.

PRA was measured by the method of Boucher et al. (9) in which an additional purification step was introduced. After neutralization of the angiotensin-containing eluate of the Dowex column, the aqueous solution is saturated with sodium chloride and subsequently extracted with butanol. Angiotensin is back-extracted from the organic layer into dilute hydrochloric acid and the acid solution evaporated under reduced pressure. The residue is taken up in albumin-containing buffer just before the bioassay is performed. The relative S.D. at level of 480 ng an-

giotensin/3 h/100 ml was 8.5% (16 determinations) and 8.0% at a level of 1100 ng/3 h/100 ml (11 determinations). Normal values for persons on diet containing about 115 mmol sodium were 189 ± 115 (1 S.D.) ng/3 h/100 ml in the supine and 589 ± 461 in the upright position (15 determinations). Urinary 17-hydroxycorticosteroids were measured by the method of Appleby et al. (4) urinary 11-hydroxy corticosteroids by the method of Mattingly et al. (28) urinary 17-ketosteroids by the method of Calkow et al. (10). Urinary sodium, potassium and creatinine were determined by routine laboratory methods (20).

Statistical methods

The differences in data between groups were tested by Student's two-sample test.

RESULTS

The effect of heparin and Ro 1-8307 on aldosterone and corticosterone secretory rates

Aldosterone secretory rates decreased during administration of heparin or Ro 1-8307 both in normal subjects and in patients with aldosteronism. On the first day of each course the secretory rate did not change significantly in 8 subjects (nos. 1–6) without demonstrable disturbances of the renin-angiotensin-aldosterone system (Table I) the mean secretory rate before treatment being 415 ± 197 (1 S.D.) against 468 ± 183 (1 S.D.) on the first day ($p > 0.1$). On the second day lower values were measured than before treatment. On the 4th or 5th day of heparin or Ro 1-8307 the secretory rates in 6 normal subjects (nos. 7–12) averaged 35% (range 16–62) of the pretreatment control values (Table I).

In patients with cardiac failure and aldosteronism due to diuretics (nos. 13–15) a steady decrease in aldosterone secretory rates was found during prolonged administration of heparin or Ro 1-8307 starting on the second day and falling to about 20% of control values (Table I).

Remarkably 4 or 5 days heparin or Ro 1-8307 treatment of normal subjects on sodium-restricted diet decreased aldosterone secretion to levels found in normal subjects on liberal sodium intake: the mean 119 ± 35 $\mu\text{g}/24$ h during administration of these drugs is though lower not significantly different from the mean value found in this laboratory in normal subjects on a dietary sodium intake of about 115 mmol ($p > 0.1$). The data of this study do not exclude the possibility that heparin or Ro 1-8307 given during longer periods might decrease the aldosterone secretory rates even more in normal subjects. The observations in patients 13 and 14

Table 1 Effect of heparin or Ro 1-8307 on aldosterone and corticosterone secretory rates in normal subjects (nos. 1-12) and patients with secondary aldosteronism (nos. 13-15) on low sodium intake (about 15 mmol daily)

Subj. no.	No. of days of treatment ^a	Aldosterone secretory rate ($\mu\text{g}/24\text{ h}$)			Corticosterone secretory rate ($\text{mg}/24\text{ h}$)		
		Before treatment	During treatment ^b	After treatment ^b	Before treatment	During treatment ^b	After treatment ^b
1	1 H	382	418 (1)	291 (2)	3.0	3.0 (1)	~4 (2)
2	1 H	665	550 (1)	368 (2)	6.9	7.1 (1)	6.3 (2)
3	1 H	638	791 (1)	757 (2)	7.6	7.1 (1)	5.1 (2)
4	1 H	370	437 (1)	49 (3)	~9	3.1 (1)	3.4 (3)
5 ^c	1 R	~37	281 (1)				
	2 R	~34	133 (2)				
6 ^c	1 R	200	339 (1)				
	2 R	180	173 (2)				
7	4 H	233	170 (4)	398 (4)			
		312					
8	5 H	345	128 (5)	546 (4)			
9	6 H	295	73 (4)	275 (4)			
		244					
10	6 H	331	137 (4)	280 (4)	5.~	3.3 (4)	6.4 (4)
		~38			3.6		
11	7 H	545	83 (5)	333 (4)	3.8	0 (5)	3.7 (4)
			70 (7)		3.0	1.4 (7)	
12	6 R	799	121 (4)	764 (4)	5.2	2.8 (4)	4.7 (4)
		690			6.5		
13 ^d	8 H	1265	587 (2)				
		1253	425 (4)				
14 ^d	9 R		197 (8)				
		525	423 (2)	70 (4)			
15 ^d		630	220 (4)	257 (8)			
			75 (9)				
15 ^d	11 H	919	200 (10)	738 (6)			

H=heparin, R=Ro 1-8307. Parentheses indicate no. of days. Aldosterone secretory rates were determined over 12-h periods. ^c Secondary aldosteronism due to diuretic treatment of cardiac failure.

with heart failure and secondary hyperaldosteronism due to diuretics illustrate that this may occur.

Corticosterone secretory rates were found to be decreased during heparin or Ro 1-8307 treatment in 3 subjects (nos. 10-12) (Table I). Values of about one half of pretreatment control values were found on the 4th-7th day of drug administration. On the first day of treatment the secretory rate did not change appreciably (nos. 1-4) (Table I).

In these studies aldosterone and corticosterone secretory rates were measured simultaneously both before and during courses of heparin or Ro 1-8307 injections. Table II summarized the percentages of inhibition of aldosterone and corticosterone secretory rates in the studies in which the drugs were given during at least 3 days. The mean percentage of inhibition of the aldosterone secretory rate was significantly higher ($p < 0.05$) than that of corticosterone.

Recovery after drug withdrawal On the 4th day after withdrawal of heparin or the heparinoid the aldosterone secretory rates in 6 normal subjects (nos. 7-12) were similar to the control values: the mean after withdrawal ($433 \pm 191 \mu\text{g}/24\text{ h}$) being not significantly different ($p > 0.1$) from the corresponding pretreatment level ($410 \pm 195 \mu\text{g}/24\text{ h}$). The corticosterone secretory rates also returned to the control values: the mean after treatment being equal to the mean before ($4.9 \text{ mg}/24\text{ h}$) (Table I).

The effects of a glutein and ACTH on the secretory rates of aldosterone and corticosterone before and during administration of heparin or Ro 1-8307. Angiotensin ($5-10 \text{ mg/kg b wt./min}$ over 24 h) did not elicit rise in aldosterone secretory rate on the 6th or 7th day of heparin administration (subjects 8 and 9). Before heparin the same amount of angiotensin increased the aldosterone secretory rate significantly ($p < 0.005$). The stimulatory effect of angiotensin was also absent on the first day of heparin or Ro 1-8307 injections in two subjects (nos. 16 and 17) without aldosteronism due to administration of

The effects of a glutein and ACTH on the secretory rates of aldosterone and corticosterone before and during administration of heparin or Ro 1-8307.

Angiotensin ($5-10 \text{ mg/kg b wt./min}$ over 24 h) did not elicit rise in aldosterone secretory rate on the 6th or 7th day of heparin administration (subjects 8 and 9). Before heparin the same amount of angiotensin increased the aldosterone secretory rate significantly ($p < 0.005$). The stimulatory effect of angiotensin was also absent on the first day of heparin or Ro 1-8307 injections in two subjects (nos. 16 and 17) without aldosteronism due to administration of

Table II Percentage inhibition of simultaneously measured aldosterone and corticosterone secretory rates during treatment with heparin or Ro 1-8307

Subj. no.	Percentage inhibition		Treatment*
	Aldosterone secretory rate	Corticosterone secretory rate	
10	52	39	Heparin (4)
1	84	40	Ro 1-8307 (4)
11	84	47	Heparin (5)
11	87	63	Heparin (7)
Mean	76.8	47.2	
		$p < 0.05$	

No. of days within parentheses.

diuretics (Table III). Fig. 1 illustrates the data of one of the subjects in detail. The natriuretic effect of heparin is evident. The BP response elicited by angiotensin was about equal before and during drug treatment.

ACTH responsiveness of the adrenals was tested in several studies (Tables IV and V). A total amount of 120 U was administered in a continuous i.v. infusion over 24 hours. In two normal subjects (nos. 5 and 6) the effect of ACTH on the secretory rate of aldosterone was tested twice, once on the 1st and on the 2nd day of a course of Ro 1-8307 injection (Table IV). Attention is drawn to the fact that observations were made in different periods of the study in order to avoid interference with the well known diminishing effect of successive doses of ACTH on aldosterone secretion (11, 22, 34).

The data of Table IV illustrate that the stimulatory effect of ACTH on the first day of treatment with heparin or Ro 1-8307 (subjects 5 and 6) was quantitatively similar to the effect of the same dose of ACTH before the administration of these drugs (subjects 10 and 12). Observations later in the study periods show that the absolute amounts of aldosterone produced in response to ACTH fall clearly after more days of heparin or Ro 1-8307. However with regard to the appropriate control values the fractional increases before and during administration of these drugs were almost equal.

Comparing the effects of angiotensin and ACTH (Tables III and IV, Figs. 1 and 2) a remarkable difference between the responses to these stimuli during heparin or Ro 1-8307 was found. At the chosen dose both stimuli increased the aldosterone secretory rate before administration of the sulphated polysaccharides by one and a half to threefold. Neither on the first nor on following days of heparin or heparinoid however was a response to angiotensin discernible, whereas during ACTH a fractional increase of the aldosterone secretory rate was found similar to that before treatment with the polysaccharides.

The data of Table V on the effect of ACTH on the corticosterone secretory rate before and during heparin or Ro 1-8307 present the same pattern as Table IV shows for aldosterone. Although the amount of corticosterone produced by stimulation with ACTH was clearly less during than before drug administration the fractional increase was

Table III Effect of angiotensin (5–10 ng/kg/min during 24 h i.v.) on aldosterone secretory rates in normal subjects and patients with secondary aldosteronism on low sodium intake (about 15 mmol daily) before and during treatment with heparin or Ro 1-8307

Aldosterone secretory rate (μ g/24 h) ^a							Treatment
Subj. no.	Control before treatment	Angiotensin before treatment	Angiotensin during 1st day of treatment	Control during 4th–5th day of treatment	Angiotensin during 6th–7th day of treatment	Control after treatment	
<i>Normal subjects</i>							
8	345 (4)	658 (6)		128 (11)	117 (13)	546 (17)	Heparin
9	295 (6)	587 (8)		73 (15)	62 (17)	275 (21)	Heparin
18	233 (5)	522 (7)					
19	485 (5)	447 (7)					
<i>Secondary aldosteronism^a</i>							
16	747 (5)	1 604 (7)	764 (10)				Heparin
17	1 100 (4)	1 548 (6)	1 154 (11)				Ro 1-8307

* Due to sodium depletion by natriuretic treatment.

Parentheses indicate no. of days of study period.

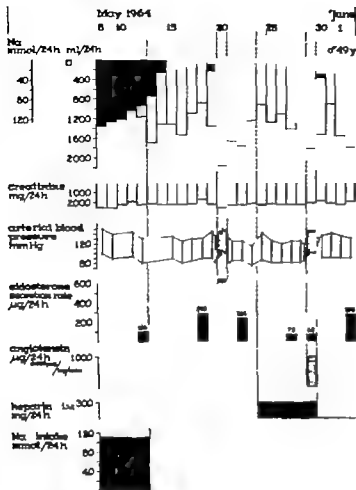


Fig. 1 Effect of angiotensin on the aldosterone secretory rate in normal subject (no. 9) on a constant 15 mmol sodium diet before and during heparin treatment. During treatment the aldosterone secretory rate decreased and sodium loss occurred. Before treatment angiotensin increased the secretory rate of aldosterone. During treatment angiotensin did not stimulate aldosterone secretion. Note that the BP response to angiotensin was about equal before and during heparin treatment.

Table IV Effect of ACTH (120 U/24 h) on aldosterone secretory rate in normal subjects on low sodium intake before and during treatment with heparin or Ro 1-8307

In subject 5 and 6 the secretory rates were determined over 12-h periods. The effect of ACTH in these subjects was studied in two separate courses of Ro 1-8307 treatment. In subject 5 the first course started on day 11 and the second on day 17. In subject 6 the start of the separate courses was on days 11 and 15.

Aldosterone secretory rate ($\mu\text{g}/24 \text{ h}$)

Subj. no.	Control before treatment	ACTH before treatment	ACTH during 1st day of treatment	ACTH during 2nd day of treatment	Control during 4th day of treatment	ACTH during 6th day of treatment	Control after treatment	Treatment
5	237 (7) 234 (12)		710 (13)	430 (18)				Ro 1-8307
6	200 (5) 180 (10)		1 097 (11)	337 (16)				Ro 1-8307
10	331 (5) 238 (10)	1 104 (7)			137 (15)	262 (18)	280 (21)	Heparin
11	799 (5) 690 (17)	1 768 (7)			121 (16)	331 (18)	764 (22)	Ro 1-8307

Parentheses indicate no. of days of study period

Table V Effect of ACTH (120 U/24 h i.v.) on corticosterone secretory rate in two normal subjects on low sodium intake before and during treatment with heparin or Ro 1-8307

Subj. no.	Corticosterone secretory rate (mg/24 h)					
	Control before treatment	ACTH before treatment	Control during 4th day of treatment	ACTH during 6th day of treatment	Control after treatment	Treatment
10	5.2	51.0	3.3	39.0	6.4	Heparin
12	5.2	104.0	2.8	45.0	4.7	Ro 1-8307

equal in both experimental situations. Fig. 2 shows one of these observations in detail (subject 12).

The effects of heparin or Ro 1-8307 on the urinary excretion of other corticosteroids than aldosterone and the effect of ACTH upon these excretions before and during administration of these drugs

In two of the subjects studied the excretory rates of both 17- and 11-hydroxycorticosteroids were meas-

ured on successive days before and during courses of heparin or Ro 1-8307. Table VI shows that the excretion of these steroids was not changed by treatment with either agent. Table VII illustrates that ACTH increased the excretory rates of both steroid groups during administration of the polysulphated polysaccharides to levels comparable with those before treatment. In addition the effect of ACTH on the excretory rate of 17-ketosteroids dur-

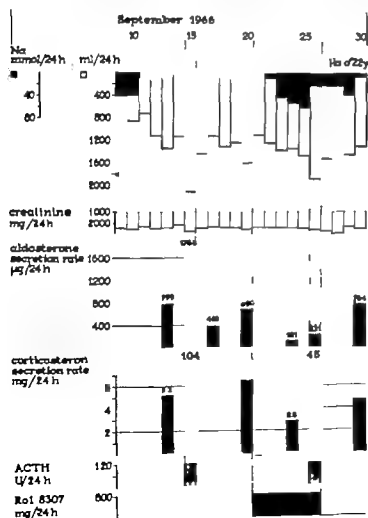


Fig. 2 Effect of ACTH on aldosterone and corticosterone secretory rates in subject 12 on constant 15 mmol sodium diet before and during treatment with Ro 1-8307. During treatment both secretory rates decreased and natriuresis occurred. The fractional increase in both aldosterone and corticosterone secretory rates by stimulation with ACTH was about equal before and during Ro 1-8307 treatment. Note that during treatment ACTH inhibited drug-induced natriuresis.

Table VI Effects of heparin or Ro 1-8307 on the excretory rates of urinary 17 and 11-hydroxycorticosteroids (mean \pm S.D.)

Subj no.	17-hydroxycorticosteroids (mg/24 h)		11-hydroxycorticosteroids (μ g/24 h)	
	Before treatment	During treatment	Before treatment	During treatment
10*	9.4 \pm 1.7 N=5	9.7 \pm 2.2 N=6	178 \pm 31 N=8	199 \pm 35 N=5
		s.		n.s.
12*	16.2 \pm 3.0 N=9	15.8 \pm 1.2 N=5	44 \pm 65 N=8	206 \pm 98 N=5
		s.		n.s.

Heparin treatment. Ro 1-8307 treatment. n.s.=not significant

ing heparin or Ro 1-8307 was almost identical to that before administration of these drugs although the control values during injections of the sulphated polysaccharides were lower than in the pretreatment period.

The effect of heparin on the activity of the renin-angiotensin system

In two male subjects with femoral vein thrombosis (nos. 20 and 21) the effect of heparin administration on PRA was studied before, during and in one individual, after administration of the drug (Table VIII). Aldosterone secretory rates were obtained on alternating days. Heparin was injected during 5 days in the first and 7 days in the second study the dose ranging from 220 to 350 mg/day. In the first study sodium loss during heparin amounted to about 330 mmol. In this period PRA did rise very slightly. After drug withdrawal however the PRA values appeared to be about twice the pretreatment level. For unknown reasons the pretreatment aldosterone se-

cretory rates in this subject were higher than those normally found during sodium restriction in this laboratory. In the second study sodium loss during the 7 days of heparin injections amounted to 270 mmol. In this observation PRA showed an equivocal decrease. In both studies the aldosterone secretory rate decreased distinctly (in 22 and 28 % of the pretreatment control values respectively).

DISCUSSION

Heparin and the chemically related polysulphated polyaccharide Ro 1-8307 inhibited, after treatment during 4-5 days the secretory rate of aldosterone in normal subjects on low sodium intake and in patients with aldosteronism to levels that are found in subjects on liberal sodium diets confirming our earlier observations (22) and those of Bailey and Ford (5). Thus adrenal inhibitory action became apparent on the second day of treatment with these drugs. The natriuretic action of these drugs is also

Table VII Effect of ACTH on the excretory rates of 17 and 11-hydroxycorticosteroids and 17-ketosteroids before and during treatment with heparin or Ro 1-8307

The control values represent the excretory rates on two successive days, ACTH values are those of the day of ACTH administration and the day thereafter

Subj no.	17-hydroxycorticosteroids (mg/24 h)				11-hydroxycorticosteroids (μ g/24 h)				17-ketosteroids (mg/24 h)			
	Before treatm		During treatm		Before treatm		During treatm		Before treatm		During treatm	
	Control	ACTH	Control	ACTH*	Control	ACTH	Control	ACTH*	Control	ACTH	Control	ACTH*
10*	10	42.9	13.3	6.7 (7)	240	4.330	210	6.010 (7)	18.1	31.6	9.5	27.1 (7)
	9	50.4	7.8	30.1	160	3.120	160	3.870	13.1	35.7	4.9	36.8
12*	19.0	87.9	16.7	81.2 (6)	220	5.820	200	2.310 (6)	13.5	29.8	7.8	22.7 (6)
	11.4	69.0	14.9	85.7	170	260	190	1.440	10.8	4.3	11.1	28.5

Heparin treatment. Ro 1-8307 treatment. Parentheses indicate no. of days of drug treatment period

Table VIII *Effect of heparin on PRA and aldosterone secretory rate*

Subj. no	PRA (ng/100 ml/3 h)			Aldosterone secretory rate (μ g/24 h)		
	Before treatment	During treatment	After treatment	Before treatment	During treatment	After treatment
20	675 (3)	700 (3)	1 125 (2)	1 267 (4)		318 (1)
	625 (5)	835 (5)	1 200 (3) 1 200 (5)	983 (2)	214 (4)	1 350 (4)
1	600 (3)	350 (2)		228 (4)		551 (11)
	400 (5)	250 (4) 400 (7)			81 (3) 75 (6)	

Parentheses indicate no. of days before, during and after treatment, respectively

delayed until the second day of treatment or even longer (13 22, 4 77 31). This inhibition of adrenal steroidogenesis is completely reversible after drug withdrawal. The same pattern of inhibition was shown for corticosterone. However the inhibition of the secretory rate of this corticosteroid appeared to be weaker than that of aldosterone (11 12).

During heparin or heparinoid treatment the responsiveness of the zona glomerulosa to ACTH—indicated by the fractional increase of the aldosterone secretory rate—was still present and comparable with the effect of ACTH in normal subjects on liberal sodium intake (22, 34). Corticosterone secretion during heparin or Ro

307 treatment was stimulated by ACTH in a similar way the fractional increase being about equal before and during administration of the sulphated polysaccharides. However exogenous angiotensin—unlike ACTH—did not stimulate aldosterone secretion discernibly during courses of heparin or heparinoid at least not at the chosen dose levels. One might argue that, if no angiotensin had been given, the aldosterone secretory rate would have been lowered further in response to continued treatment with heparin or its congener so that lack of response to angiotensin could be the result of inhibition by a polysulphated polysaccharide and stimulation by angiotensin. Arguing against such an interpretation is the fact that, during treatment with heparin or Ro 1-8307 aldosterone secretory rates were never lower than those found in normal subjects on liberal sodium intake, as is illustrated by the values of subjects 8 and 9 in Table III. At this point it should be added that in two observations not reported in this paper the effect of similar amounts of angiotensin was studied in two subjects on liberal salt intake. Angiotensin increased aldosterone secretory rates in these two subjects from 94 and 150

μ g/24 h to 440 and 228 μ g, respectively. Although the number of observations is too small to permit definite conclusions these data suggest that heparin and Ro 1-8307 inhibit selectively the action of angiotensin in the zona glomerulosa, whereas ACTH remains stimulatory with the zona glomerulosa working at a lower gear.

From the few observations reported in the literature it appears that during heparin or heparinoid treatment PRA increases (5 30). From these and the two studies reported here in which no consistent effect on PRA during heparin treatment was observed it is concluded that aldosterone inhibition by heparin is clearly not mediated by renin suppression.

So far no indication of interference with cortisol production has been found. The excretory rate of 17 and 11-hydroxycorticosteroids did not change during heparin or Ro 1-8307 treatment in accordance with earlier reported data (1 27 37). These findings per se do not rule out partial inhibition of the zona fasciculata function as compensatory release of ACTH might rapidly abolish such an inhibitory effect (18). The results of an earlier study (27) in which urinary 17-OH-corticosteroid excretion, stimulated by ACTH, did not change distinctly during addition of heparin but rose significantly when heparin was omitted and ACTH continued do suggest that under special circumstances heparin can influence the function of the zona fasciculata. In fact the decrease in urinary excretion of 17-ketosteroids as reported earlier (26, 36) and reconfirmed by the observations in this study could mean that heparin and Ro 1-8307 interfere with adrenal function in a rather unspecific way. However our observation that exogenous ACTH increased the excretory rates of 17-hydroxycorticosteroids, 11-hydroxylated compounds and 17-ketosteroids during drug treatment to

the same extent as in the pretreatment period suggests that the main effect of short-term administration of heparin or Ro 1-8307 is confined to the zona glomerulosa. This explanation is in accordance with the histological findings in rats treated with these drugs. Valfert et al. (35) reported a decreased width of the zona glomerulosa of salt-depleted rats after administration of heparin. Abbott et al. (2, 3) found atrophy of the zona glomerulosa of rats on normal sodium intake without clearcut alterations in the zona fasciculata after treatment with Ro 1-8307 and Chremos et al. (14) obtained identical results in regenerating rat adrenal glands and in intact adrenals of sodium-deprived rats.

In this context the observation by Wilson and Goetz (39) may be mentioned. These authors reported isolated hypoaldosteronism and at post mortem examination, atrophy of the adrenal zona glomerulosa in a 38-year-old man who was treated for severe arteriosclerosis and hypercholesterolemia with intramuscular heparin injections during 4 years.

Data of *in vitro* studies indicate that heparin can interfere with the biosynthesis of aldosterone. Sharma et al. (33) studied the conversion of labeled corticosterone into aldosterone in a mitochondrial preparation of frog adrenals. These investigators found that, in the absence of calcium in the incubation medium, heparin inhibits this conversion in rather low concentrations (10^{-6} mol). Benraad et al. (unpublished observations) studied the conversion of labeled progesterone into corticosteroids by slices of an aldosteronoma and by beef and rat adrenals. At a higher concentration (10^{-4} mol) than used by Sharma et al. heparin caused a distinct inhibition of the conversion into corticosterone, 18-hydroxycorticosterone and aldosterone with concomitant accumulation of deoxycorticosterone. Both these *in vitro* studies indicate that heparin or Ro 1-8307 interferes with the biosynthesis of aldosterone, the latter providing additional evidence that also corticosterone synthesis is inhibited by these drugs. Together these findings might reflect our results in clinical studies, showing inhibition of biosynthesis of both aldosterone and corticosterone.

The combination of unresponsiveness of the zona glomerulosa to angiotensin with maintenance of responsiveness of ACTH found during heparin or Ro 1-8307 treatment, is rather unique effect of an adrenal inhibitory drug. Angiotensin as well as ACTH are considered to be physiological regulators

of mineralocorticoid production (38). Their relative importance for aldosterone secretion in man is still a matter of dispute and their mechanisms of action are still unclear. Polysulphated polysaccharides such as heparin or Ro 1-8307 might be useful tools in the unraveling of these problems.

ACKNOWLEDGEMENT

This investigation was supported by grant from the Netherlands Organization for the Advancement of Pure Research (Z.W.O. Fungo).

REFERENCES

- Abbott, E. C., Gornall, A. G., Sutherland, D. J. A., Stiefel, M. & Laidlaw, J. C. The influence of heparin-like compound on hypertension, electrolytes and aldosterone in man. *Canad. med. Ass. J.* 94: 1155 1966.
- Abbott, E. C., James, V. H. T., Parker, R. A., Pearl, W. S. & Fraser, R. The effect of sulphated monopolysaccharide (Ro 1-8307) on adrenal and kidney morphology and function in the rat. *Hormones* 3: 129 1972.
- Abbott, E. C., Monkhouse, F. C., Steiner, J. W. & Laidlaw, J. C. Effect of sulfated monopolysaccharide (Ro 1-8307) on the zona glomerulosa of the rat adrenal gland. *Endocrinology* 78: 631 1966.
- Appleby, J. I., Gibson, G., Norynberkin, J. K. & Stubbs, R. D. Indirect analysis of corticosteroids. I. The determination of 17-hydroxycorticosteroids. *Biochem. J.* 60: 453 1955.
- Bally, R. E. & Ford, H. C. The effect of heparin on sodium conservation and on the plasma concentration, the metabolic clearance and the secretion and excretion rates of aldosterone in normal subject. *Acta endocr. (Kbh.)* 60: 249 1969.
- Benraad, Th. J. *Heparing van aldosteron met behulp van een dubbelschoot methode*. Thesis, University of Nijmegen, The Netherlands 1966.
- Benraad, Th. J. & Kloppenburg, P. W. C. Double isotope assay of aldosterone in urinary extracts with the combined use of thin-layer and paper chromatography. *Chin. chim. Acta* 1: 365 1965.
- Plasma renin activity and aldosterone secretory rate in man during chronic ACTH administration. *J. clin. Endocr.* 31: 581 1970.
- Boucher, R., Veyral, R., de Champlain, J. & Genest, J. New procedures for measurement of human plasma angiotensin and renin activity levels. *Canad. med. Ass. J.* 90: 194 1964.
- Callow, N. H., Callow, R. K. & Emmert, C. W. Colorimetric determination of substances containing the group $-\text{CH}_2\text{CO}-$ in urine extracts as an indication of androgen content. *Biochem. J.* 7: 1312, 1938.
- Casparie, A. F. *De secretiemethode van corticosteron bij de mens*. Thesis, University of Nijmegen, The Netherlands 1968.

12. Casparie, A. F. Bernad, Th. J. Kloppenborg, P. W. C. & Majoor C. L. H. Effect of heparin on the corticosterone secretion rate with a description of the double isotope method used (Abstract). *Acta endocr (Kbh.)*, Suppl. 119: 140 1967
13. Cefka, V. de Vries L. A. Smorenberg-Schoorl, M. E., van Damselaar J. J., Borst, J. G. G. & Majoor C. L. H. Effect of heparinoid and spironolactone on the renal excretion of sodium and aldosterone. *Lancet* 1 317 1960
14. Chremos, A. N. Laidlaw J. C. & Rosa, J. L. Effect of a sulfated macopolysaccharide (Ro 1-8307) on the zona glomerulosa of regenerating rat adrenal glands and of intact glands in sodium-deprived rats. *Endocrinology* 85 337 1969
15. Conn J. W. Rovner D. R., Cohen, E. L. & Anderson, J. E. Jr. Inhibition by heparinoid of aldosterone biosynthesis in man. *J. clin. Endocr* 26: 577 1966
16. Conn, J. W. Rovner D. R., Cohen, E. L. & Kleinberg, S. A block in adrenal biosynthesis of aldosterone induced by heparinoid (Abstract). *Clin. Res.* 12: 351 1964
17. Ehrlich, E. N. Heparinoid-induced inhibition of aldosterone secretion in pregnant women. The role of augmented aldosterone secretion in sodium conservation during normal pregnancy. *Amer. J. Obstet. Gynec.* 109: 963 1971
18. Fishman, L. M. Liddle G. W. Island, H. P. Flierischer N. & Kischel, D. Effects of amino-glucosaminide on adrenal function in man. *J. clin. Endocr* 27: 481 1967
9. Ford, H. C. & Bailey R. E. The effect of heparin on aldosterone secretion and metabolism in primary aldosteronism. *Steroids* 7 30 1966
- Gorier, E. & de Graaff W. C. Klinische diagnose. Stenfort Kroese N. V. Leiden 1953
21. Kikman, B. Rebill, J. & Bartler F. C. Alterations in aldosterone secretion during sustained maximal ACTH therapy. *Progr. 43rd Annual Meeting of the Endocrine Society* 75 1961
22. Kloppenborg, P. W. C. De secretie van aldosteron onder normale en pathologische omstandigheden. Thesis University of Nijmegen. The Netherlands 1966
23. Kloppenborg, P. W. C. Bernad, Th. J. & Majoor C. L. H. Metingen van de secretiesnelheid van aldosteron (Abstract). *Ned. T. Geneesk.* 109 1178 1965
24. Majoor C. L. H. Waarnemingen over de excretie en de secretie van aldosteron en andere bijverschorssteroiden tijdens toediening van heparine en heparinoiden en bij patiënten met decompensatie cordis. *Ned. T. Geneesk.* 113 767 1969
25. Majoor C. L. H., Preesen, H., van Munster P. J. J. & Schlattmann, R. J. A. F. M. Het diuretische effect van heparine in het bijzonder bij patiënten met het nefrotische syndroom. *Ned. T. Geneesk.* 101 1301 1957
26. Majoor C. L. H. Schlattmann, R. J. A. F. M., Jansen, A. P., Frohn, H. & Preesen, H. Investigations on the influence of heparin and some heparinoids upon the renal excretion of sodium and adrenal steroids—especially aldosterone—in man and dog. *Folia med. Neerl., Additamentum* 1 40 1962
27. Majoor C. L. H. Schlattmann, R. J. A. F. M., Jansen, A. P. & Preesen, H. Excretion pattern and mechanism of diuresis induced by heparin. *Clin. chin. Acta* 5 391 1960
28. Mattingly D., Dennis, P. M., Pearson, J. & Cope C. L. Rapid screening test for adrenal cortical function. *Lancet* 2. 1046, 1964
29. Peterson, R. E. & Pierce C. E. The metabolism of corticosterone in man. *J. clin. Invest.* 39: 741 1960
30. Rose J. L. Price C., Schiefel, M. & Laidlaw J. C. The influence of glucocorticoid and heparin on aldosterone production. In: *Hypertension* 72 (ed J. Genest and E. Koiv), p. 326. Springer Verlag Berlin Heidelberg and New York 1972
31. Schlattmann R. J. A. F. M., Jansen, A. P., Preesen, H., van der Korf, J. K. & Majoor C. L. H. The natriuretic and aldosterone-suppressive action of heparin and some related polysulfated polymericarides. *J. clin. Endocr* 4 35 1964
32. Schlattmann, R. J. A. F. M., Preesen H., Jansen A. P. & Majoor C. L. H. The natriuretic action of heparin and some related substances. *Lancet* 1 314 1960
33. Sharma, D. C., Norenberg, C. A. & Dorfman, R. L. Studies on aldosterone biosynthesis in vitro. II. Biochemistry 6 3472, 1967
34. Tucci J. R., Espiner E. A., Jagger P. I., Paul, M. L. & Lamer D. P. ACTH-stimulation of aldosterone secretion in normal subjects and in patients with chronic adrenocortical insufficiency. *J. clin. Endocr* 27 568, 1967
35. Vallent, K., Fachel, J., Palkovits, M. & Dévényi, L. Über die Wirkung der Heparinbehandlung auf das histologische Bild der Nebennierenrinde und auf den Index der juxtaglomerulären granulierten Zellen im Nierengewebe. *Z. Zellforsch.* 63 728 1964
36. Vallent, K., Feher T. & Fachel, J. Über die Wirkung der Heparinbehandlung auf die Ausscheidung der einzelnen 17-Ketosteroidfraktionen. *Endokrinologie* 48 51 1965
37. Veyrat, R., Manning, E. L., Fabre J. & Müller A. F. Mesure de la sécrétion de l'aldostérone sous administration d'un adrénostatique semi-synthétique l'héparinoïde Ro 1-8307. *Rev. Franç. Etudes Clin. et Biol.* 8 667 1963
38. Williams, O. H. & Dhuhy R. G. Aldosterone biosynthesis. Interrelationship of regulatory factors. *Amer. J. Med.* 33: 594 1972
39. Wilson, I. D. & Goetz, F. C. Selective hypokalaemia after prolonged heparin administration. *Ame. J. Med.* 36 635, 1964

INSULIN RELEASE IN FASTING MAN INDUCED BY IMPURE BUT NOT BY PURE PREPARATIONS OF CHOLECYSTOKININ

Pavo Hedner, Gunnar Persson and Dag Ursing

From the Department of Internal Medicine, University Hospital, Lund, Sweden

Abstract. Most preparations of cholecystokinin reported to release insulin have been impure. When highly purified preparations of extracted cholecystokinin and also the synthetic C-terminal octapeptide of the hormone became available for use in humans, we investigated their insulinotropic activity in comparison with a crude preparation of cholecystokinin in 10 fasting non-diabetic subjects. The doses employed were 75 Ivy dog units, except for the synthetic C-terminal octapeptide of cholecystokinin that was given in a dose of 200 Ivy dog units to compensate for a shorter half-life. Blood samples for determination of insulin and glucose were drawn every minute during 10 min after each injection, thereafter at intervals of 5 min. The mean plasma insulin level increased significantly reaching peak 4-5 min after i.v. injection of the crude cholecystokinin preparation, but after the other two preparations the plasma insulin level was not significantly changed. The blood glucose level was not significantly changed by any of the preparations used. It is concluded that the plasma insulin peak seen in man after v. injection of the less highly purified preparation was due not to cholecystokinin but to some other agent present in this less pure preparation. The identity of this factor is discussed.

However, the reports on the insulinotropic activity of cholecystokinin in man are contradictory. Thus Raptis *et al.* (18) and Adlmg and Utigerann (1) found a plasma insulin peak after i.v. administration of cholecystokinin, while Dupré and Beck (8) found no such effect of the hormone. The results of these investigations may however be difficult to interpret due to the use of impure preparations of cholecystokinin and recently Creutzfeld (5) stressed the importance of using pure and homogenous preparations when investigating the insulinotropic effect.

We had the opportunity to obtain pure and well defined preparations of cholecystokinin, namely a highly purified extracted preparation, and the synthetic C-terminal octapeptide of the hormone. Therefore we investigated the ability of these preparations of cholecystokinin to release insulin in man as compared to a less highly purified preparation.

MATERIAL AND METHODS

Ten non-diabetic subjects, six men and four women, aged 20-45 years, were investigated.

The subjects had fasted overnight. On the following morning, cubital vein of each arm was cannulated using indwelling plastic catheters with stopcocks. One of the catheters was used for injection of the test substances, the other one for blood sampling. The catheters were perfused with saline after each blood sampling.

The three test preparations were given i.v. at intervals of 30 min. 5 ml blood were drawn into test tubes containing EDTA to prevent coagulation 15 and 5 min before the first injection. After the injection of test substance had begun, 5 ml blood were drawn every minute for 10 min, and also at 15, 20, 25 and 30 min after the beginning of the injection.

From every second blood sample 0.1 ml was taken immediately after collection for glucose determination by the method of Mark (15). The remainder of each blood

In 1964 McIntyre *et al.* (16) demonstrated that hyperglycemia produced by oral glucose caused a greater release of insulin in man than a comparable degree of hyperglycemia produced by i.v. glucose. In the same year Dupré (6) found that a crude extract of intestinal mucosa improved i.v. glucose tolerance. These observations drew the attention to the possibility that during the resorption of glucose the gastrointestinal tract might produce a humoral factor that enhanced the release of insulin from the β -cells. The structure of this insulinotropic hormone is still not known, but an ability to release insulin has been ascribed to several of the known gastrointestinal hormones including cholecystokinin-pancreozymin.

sample was centrifuged within 10 min of collection and the plasma separated and frozen for later determination of insulin by a double antibody radioimmunoassay (Phadbas Insulin Test, Pharmacia, Sweden). The following test substances were administered:

1) Cholecystokinin extracted from hog mucosa, containing 1 900 Ivy dog units/mg, obtained in sterile ampoules from the GIH Laboratory Karolinska Institutet, Stockholm, Sweden. Seventy-five Ivy dog units were given i.v. during 1 min to each subject.

2) Essentially pure cholecystokinin extracted from hog intestinal mucosa, containing 3 000 Ivy dog units/mg, supplied by Professor V. Mott the GIH Laboratory Karolinska Institutet, Stockholm. The extract was dissolved in 0.9% NaCl and sterile filtered using Millipore filters which had been previously passed by a solution of protamine sulphate to saturate the protein-binding capacity of the filter. The sterile solution of cholecystokinin was tested *in vitro* and it was found that no cholecystokinin activity had been lost in the sterile filtration procedure. Seventy-five Ivy dog units (25 µg) were given i.v. during 1 min to each subject.

3) The synthetic C-terminal octapeptide of cholecystokinin supplied by Dr M. A. Osdertl, the Squibb Institute for Medical Research, New Brunswick, N.J. USA, was dissolved in 0.9% NaCl to a concentration of 0.33 µg/ml and sterile filtered as described above. By an *in vitro* assay using the guinea pig gallbladder it was ascertained that the biological activity was not reduced by the filtration procedure. Of this solution 7 ml were administered i.v. to each subject during the first minute, followed by 3 ml/min until a total dose of 20 ml was reached, corresponding to 700 Ivy dog units of cholecystokinin.

The test substances were given in the order 2, 1 to four subjects in the order 2, 3, 1 to five and in the order 1, 2, 3 to subject 4. A randomized order of administration of the three preparations was thus not adopted. Instead the inactive preparations were given first to all but one subject, as it was suspected that an active preparation might reduce the effect of preparations to follow within such a short period as 30 min (13).

RESULTS

The administration of 75 Ivy dog units of partially purified cholecystokinin (1 500 Ivy dog units/mg) was followed by an increase in the plasma insulin concentration in all subjects. The mean plasma insulin concentration in the group was significantly above the control level ($p < 0.05$) from the 2nd to the 8th min after the beginning of the injection and then gradually returned to the control level (Fig. 1). A peak was seen between the 4th and 5th min after the beginning of the injection with a mean value of 33 µU/ml (Fig. 1).

Neither the synthetic C-terminal octapeptide of cholecystokinin nor the essentially pure preparation of the hormone (3 000 Ivy dog units/mg) produced

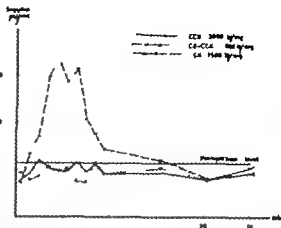


Fig. 1 Mean plasma insulin levels in 10 subjects following 1. administration of cholecystokinin extracts containing 1 900 and 3 000 Ivy dog units/mg, respectively and the synthetic C-terminal octapeptide of cholecystokinin (CCK-CKA). Standard deviations are given in the text.

any significant change in the plasma insulin level in the group (Fig. 1).

The standard deviation of the insulin concentration in the group was 4.2 and 5.3 µU/ml respectively for the two preinjection samplings between 3.1 and 7.5 µU/ml following the injection of the inactive substances and between 4.1 and 16.8 µU/ml following the injection of the partially purified preparation of cholecystokinin.

None of the test substances caused any significant change in the blood glucose level. Apart from slight abdominal discomfort for 1–2 min the subjects experienced no side-reactions to any of the test substances.

DISCUSSION

The synthetic C-terminal octapeptide of cholecystokinin used in the present investigation possesses all the effects of the whole hormone molecule on gallbladder spincter of Oddi, exocrine pancreas and small intestine (11–19). As the C-terminal octapeptide of cholecystokinin seems to be inactivated at a higher rate than extracted cholecystokinin (19) a larger dose in Ivy dog units was administered over a longer period. This should guarantee a sufficient cholecystokinetic effect judged from an earlier investigation of this preparation (12). In spite of this the C-terminal octapeptide of cholecystokinin failed to release insulin in the subjects investigated. To check the possibility that

an insulin-releasing effect might be bound to another part of the cholecystokinin molecule a preparation of extracted hormone considered to be practically 100% pure was in estigated. Again there was no effect on the plasma insulin concentration.

Our observations indicate that cholecystokinin-pancreozymin is of minor importance for the release of insulin in man. They are supported by an investigation by Young et al. (21) who found that plasma pancreozymin levels rose only after a very large oral glucose load and the increase of pancreozymin then occurred later than that of insulin. The same highly purified cholecystokinin extract as was used by us has been given to rats by Dupré (7) and to man by Rabinovitch and Dupré (17) without any significant change in the plasma insulin level.

Bertaccini et al. (2) found that coerulein produced an increased plasma insulin level in dogs. Coerulein is a decapeptide derived from frog skin but possessing a C-terminal structure identical to that of the C-terminal octapeptide of cholecystokinin except for one amino acid. The insulinotropic effect of this substance is not in line with the results of the present investigation but may be explained by species differences as Unger et al. (20) found that a pancreozymin preparation of a purity comparable to the purest used in the present investigation released insulin and glucagon in dogs.

The insulinotropic activity in man following administration of cholecystokinin preparations of low purity which was found in many investigations (1, 18), was verified in the present investigation which however provides evidence that the insulinotropic activity should be ascribed to the impurities rather than to the cholecystokinin content in these preparations.

The identity of the insulin-releasing factor present in the impurities of some cholecystokinin preparations is not known. However it might be expected to belong to the glucagon-secrelin group of intestinal hormones that has a well established insulinotropic effect (9, 14). Brown et al. (4) have isolated a substance named gastric inhibitory peptide from an impure preparation of cholecystokinin. The gastric inhibitory peptide has structural similarities with glucagon (3). It has shown a high insulinotropic activity in preliminary experiments in man (10). The gastric inhibitory peptide should however be extensively removed from a cholecystokinin extract of the purity used in the present investigation (1 500 Ivy dog units/mg) (4). It is possible that the gastric

inhibitory peptide is not the only insulin-releasing factor present in partially purified preparations of cholecystokinin.

REFERENCES

- Adlung J & Uthgenenst, H. Über den Einfluss von Pancreozymin auf Blutzucker sowie, ^{14}C Glukoseumsatz und -Oxidation. *Z. Gastroint.* 9: 589 1971.
- Bertaccini, O. De Caro, H. & Melchiorri, P. The effects of coerulein on insulin secretion in anesthetized dogs. *Brit. J. Pharmacol.* 40: 78 1970.
- Brown, J. C. & Dryburgh, J. R. A gastric inhibitory polypeptide II. The complete amino acid sequence. *Canad. J. Biochem.* 49: 867 1971.
- Brown J. C. Muir, V. & Pederson R. A. Further purification of polypeptide demonstrating enterogastrone activity. *J. Physiol. (Lond.)* 209: 97 1970.
- Crewsfield W. Gastrointestinal hormones and insulin secretion. *New Engl. J. Med.* 288: 138 1973.
- Dupré, J. An intestinal hormone affecting glucose disposal in man. *Lancet* 2: 677 1964.
- Effects of gastrointestinal hormones on the disposal of nutrients. Symposium on gastrointestinal hormones—regulation, secretion and actions. Presented at the 14th International Congress of Endocrinology Washington, D.C. June 18–4 1977.
- Dupré, J. & Beck, J. C. Intestinal hormones and plasma-insulin. *Lancet* 1: 279 1966.
- Dupré, J., Rojas, L., White J. J., Unger R. H. & Beck, J. C. Effects of secretin on insulin and glucagon in portal and peripheral blood in man. *Lancet* 1: 26 1966.
- Dupré J., Ross, S. A., Watson, D. & Brown, J. C. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J. clin. Endocr.* 7: 826 1977.
- Hedner P. Effect of the C-terminal octapeptide of cholecystokinin on guinea pig ileum and gall-bladder in vitro. *Acta physiol. scand.* 78: 132, 1970.
- Hedner P. & Landerberg, A. Use of the C-terminal octapeptide of cholecystokinin for gallbladder evacuation in cholecystography. *Amer. J. Roentgenol.* 116: 320 1972.
- Isaac R., Navez, M. P., Ardalion R. & Meyner A. Insulin response to glucose and secretin in uremic and normal subjects. *Europ. J. clin. Invest.* 2: 475 1972.
- Jarrett, R. J. & Cohen N. M. Intestinal hormones and plasma-insulin. Some observations on glucagon, secretin and gastrin. *Lancet* 2: 861 1967.
- Marks V. An improved glucose-oxidase method for determining blood C.S.F. and urine glucose levels. *Clin. chim. Acta* 4: 395 1949.
- McIntyre N., Holdsworth, C. H. & Turner D. S. New interpretation of oral glucose tolerance. *Lancet* 1: 20 1964.
- Rabinovitch A. & Dupré J. Insulinotropic and

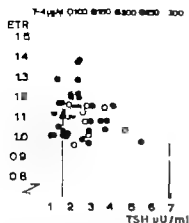


Fig. 1 ETR and TSH in hypothyroid patients during euthyroidism caused by *l*-thyroxine replacement treatment. T-4=*l*-thyroxine dosage.

bound iodine, absolute free thyroxine and TSH determinations. The cause of hypothyroidism was surgical thyroidectomy in 16 cases, ^{131}I iodine treatment for thyrotoxicosis in 5, serotonergic thyroiditis without goitre in 22 and with goitre in 5 cases. The assessment of euthyroidism was done with the aid of a stable clinical normalization with no symptoms or signs of hyperthyroidism and with no need in change the daily dose of T-4 for at least 2 months. The clinical situation was assessed according to recognized principles (5).

The TSH measurements were made by the Medix Clinical Laboratory by radioimmunoassay (4).

The ETR tests were made by the Res-O-Mix® ETR™ Test (Mallinckrodt Chemical Works, St. Louis, Missouri, USA) as described by the manufacturer as well as by various investigators (9, 10, 11, 13, 15). After denaturation of the proteins of the serum sample with alcohol, the separated patient thyroxine and an aliquot of patient serum are introduced into buffered solution of reference thyroxine-binding globulin labelled with ^{125}I -thyroxine. The procedure displaces labelled thyroxine from the reference thyroxine-binding globulin and the labelled thyroxine is, in turn, bound to free binding sites in the patient serum. The unbound labelled thyroxine is taken up by a resin strip and removed from the system. The amount of remaining labelled thyroxine is counted and the result is compared to that obtained with a similarly treated standard serum sample. The ETR is expressed as counts per minute standard serum/counts per minute patient serum. The euthyroid range is 0.86–1.13.

In our laboratory the radioactivity of the labelled reference thyroxine-binding globulin was checked for uniformity in every batch before use. The standard samples were always run in duplicate one at the beginning, one at the end of each series of determinations. The accuracy of the tests in multiple determinations from the same serum was $\text{ETR} \pm 0.03$.

RESULTS

The results are presented in Table I and Fig. 1. All the ETR values are within or above the normal range of 0.86–1.13 and all the TSH values are within or below the normal range of 1.6–6.9 $\mu\text{U/ml}$. The range of ETR values was 0.97–1.38 (mean 1.080) and of TSH values 1–5.5 $\mu\text{U/ml}$ (mean 2.36). The range of T-4 dosage was 100–300 $\mu\text{g/day}$ (mean 182).

DISCUSSION

In the evaluation of substitution treatment for hypothyroidism it is important that the patient's clinical improvement is the main guide-line to therapy. It is necessary however that laboratory tests are used to supplement this guide-line. In the choice of a laboratory test, not only its physiological implications but also its technical procedure must be considered. It is generally agreed that in primary hypothyroidism a TSH decrease towards normal is the most reliable sign of commencing substitution. The TSH method is not, however, suitable for routine work. Measurements of total thyroxine can be routinely used, but are not reliable when protein variations must be considered. The same is true for the triiodothyronine-resin test. A combination of the two latter tests eliminates protein variation effects but is tedious because two separate test procedures have to be performed. The ETR, which is not disturbed by protein variations, is done in a single test procedure and can be done in every laboratory where radioactivity measurements are made has definite advantages compared with the other tests.

Table I Number of patients in the different ETR and TSH ranges and dosage groups

+ = above, — = below normal range N = normal

		<i>l</i> -thyroxine ($\mu\text{g/day}$)				
		100	150	200	250	300
ETR N						
TSH N	25	5	7	8	5	
ETR N						
TSH —	4	1	2		1	
ETR +						
TSH N	16	4	4	4	1	3
ETR +						
TSH —	3			1	1	1

In the present investigation the mean dose of T-4 is in accordance with recent observations in similar patients (12). The dosage of T-4 affects the laboratory values only slightly and the dose required to obtain euthyroidism varies as shown in Fig. 1. The higher doses of T-4 (250 or 300 µg/day) are, of course concentrated in the high ETR and normal or low TSH range. On the other hand many medium doses of T-4 (200 µg/day) are seen in the normal ranges of TSH and ETR, and even in the high-normal range of TSH. The low doses are more scattered. This may be due to several reasons. Variations in absorption of T-4 from the gastrointestinal tract in different patients may play a role. The occurrence of patients with low doses of T-4 in the high ETR range may favour this view. On the other hand some patients obviously need high thyroxine concentrations to be kept euthyroid which is shown by the fact that ETR values up to nearly 1.4 may be necessary for clinical euthyroidism in some patients. Similar observations have been made with other laboratory methods. The cause of this phenomenon may for instance, be variations in the metabolism of thyroxine as well as peripheral utilization of thyroid hormones. It is obvious that T-4 treatment in hypothyroidism cannot be given in standardized dosage.

Both the TSH and the ETR are useful to exclude undertreatment with T-4 in primary hypothyroidism. They must however both be supplemented by a careful clinical evaluation if over dosage is to be avoided, as the range of values in both methods is wide for euthyroid patients. This clinical evaluation is necessary for the determination of the optimal dose and cannot at present be replaced by any available single laboratory method or combination of methods.

In the present material TSH determinations do not offer advantages compared with the ETR in the monitoring of the dose of T-4. The simpler and more rapid ETR measurement may thus well be used in such monitoring.

REFERENCES

1. Abrams C., Vagenakis, A., Azizi, F., Portnay G. & Braverman, L. A single method for measuring total thyroxine and free thyroxine index in serum. *J. nucl. Med.* 14: 740 1973.
2. Evered D. C., Ornston, B. J., Smith, P. A., Hall, R. & Bird, T. Grades of hypothyroidism. *Brit. med. J.* 1: 657 1973.
3. Evered, D. C., Young, E. T., Ornston B. J., Menzies R., Smith P. A. & Hall, R. Treatment of hypothyroidism: A reappraisal of thyroxine therapy. *Brit. med. J.* 3: 131 1973.
4. Gordin, A. & Särninen, P. Methodological study of the radioimmunoassay of human thyrotrophin. *Acta endocr. (Kbh.)* 71: 11 1972.
5. Howarth, P. N. J. & Ward, R. L. The T_4 -free thyroxine index as a test of thyroid function of first choice. *J. clin. Path.* 25: 259 1972.
6. Johnston, M. A., Squires, A. H. & Parquharson R. F. The effect of prolonged administration of thyroxine. *Ann. Intern. Med.* 35: 1008, 1951.
7. Kahn, A. Serum thyroxine levels in patients receiving L-thyroxine suppression or replacement therapy. *Canad. med. Ass. J.* 109: 279 1973.
8. Kjelstrup J. Free and total thyroxine in serum. *Scand. J. clin. Lab. Invest.* 32: 227 1973.
9. Kälendörf, K., Siestbeck-Nielsen, K., Møhlhøj Hansen, J. & Friis, T. Klinisk vurdering af en ny thyroideafunktionsundersøgelse: effektiv thyroideafunktion (ETR). *Ugeskr. Læg.* 136: 246, 1974.
10. Mickey E. K., Thorson S. C., Brown, J. L., Morrison, R. T. & McInosh, H. W. A new parameter of thyroid function—the effective thyroxine ratio. *J. nucl. Med.* 13: 165 1972.
11. Rudorff K. H., Herrmann, J. & Krieskemper H. L. Zur Methodik der Bestimmung der ETR (Effective Thyroxine Ratio). *Zsch. klin. Chem. Klin. Biochem.* 11: 259 1973.
12. Stock, J. M., Serks, M. I. & Oppenheimer J. Replacement dosage of L-thyroxine in hypothyroidism. *New Engl. J. Med.* 290: 529 1974.
13. Thorson, S. C., Mickey E. K., McInosh, H. W. & Morrison, R. T. Evaluation of new in-vitro blood test for determining thyroid status. The effective thyroxine ratio. *Brit. med. J.* 2: 67 1977.
14. Timonen, T., Haahtela, H., Reimä, M. & Vuopala, U. The value of the effective thyroxine ratio compared with other common thyroid tests. *Acta med. scand.* 195: 11 1974.
15. Welby M. L., O'Halloran, M. W. & Marshall, J. A comparison of effective thyroxine ratio and free thyroxine in serum. *Clin. chem. Acta* 43: 255 1973.

STUDIES OF URINARY BLADDER DYSFUNCTION IN AMYLOIDOSIS WITH POLYNEUROPATHY

Rune Andersson¹ and Per Björk

From the Departments of Medicine and Clinical Physiology, University Hospital, Umeå, Sweden

Abstract Eight male patients with amyloid polyneuropathy seven of whom had symptoms of dysfunction of the urinary bladder were studied by cystometry and micturition analysis and compared with eight male controls. Most of the patients with amyloidosis had an increased bladder capacity. The need to micturate was reduced, and urine retention was common. In most of these detrusor function could not be demonstrated. The maximal flow rate was usually diminished and the resistance to flow elevated. In some of the patients there was increased rigidity of the bladder wall.

In primary amyloidosis with polyneuropathy progressive sensory and motor disturbances are major manifestations of the disease. Disturbances of motility in the gastrointestinal tract, postural hypotension, abnormal sweating, impotence and disturbances of micturition point to widespread involvement of the autonomic nervous system. Earlier descriptions of the disease mention incontinence regarded as an expression of sphincter dysfunction (4-5). As was shown in a previous study of patients in northern Sweden difficulty in emptying the bladder and infection of the urinary tract were frequent findings (3). Histopathological examination revealed amyloid deposits in the blood vessels and nerves of the bladder. Amyloid deposits were also present in the detrusor musculature. The aim of the present investigation was to study the disturbance of micturition that develops in these patients by means of cystometry and micturition analysis. To our knowledge there has been no previous detailed analysis of the disturbed micturition appearing in patients with this type of amyloidosis.

Present address: Department of Medicine Örnskölvsvik Hospital, S-891 02 Örnskölvsvik, Sweden

MATERIAL

The material consisted of 8 male patients admitted to the Department of Medicine, University Hospital, Umeå. All had symptoms and signs of polyneuropathy most prominently in the lower extremities (Table I). The clinical signs pointed to involvement of the peripheral nerves and included sensory disturbances, muscle atrophy, flaccid paralysis, and loss of reflexes. The diagnosis of amyloidosis was confirmed by histological examination of biopsy material. The mean age of the patients at the time of investigation was 52 years (range 35-64). The cases are designated by capital letters as in earlier reports (1, 2, 3). In the familial cases a numeral has been added. The composition of the material is shown in Table I.

The control group consisted of 8 men admitted to the Department of Medicine, University Hospital, Umeå. Data concerning the controls are given in Table II. Their mean age was 46 years (range 37-58). None had symptoms or signs of peripheral neuropathy nor did they have any symptoms of disturbed micturition.

METHODS

Clinical grading of neuropathy

The diagnostic criteria of polyneuropathy and the clinical grading of the peripheral neuropathy and of the gastrointestinal disturbances have previously been described (1, 2): + = slight, ++ = moderate, +++ = marked disturbances. Symptoms of bladder dysfunction impotence and rectal incontinence, if present, are not graded but merely indicated by +.

Cystometry and micturition analysis

The technique used is a modification of methods previously described (8, 12). Details of the procedure are given elsewhere (7). Two teflon catheters were inserted into the urinary bladder by suprapubic puncture. A third catheter was passed through one nostril and advanced into the stomach. The end of the catheter in the stomach was connected with a thin-walled latex balloon, partially filled with air. One of the bladder catheters was connected to an infusion apparatus con-

Table I. Data on 8 male cases of primary amyloidosis with polyneuropathy

Case	Age at examination (y)	Duration of neuropathy (y)	Neuropathy ^a		Gastro-intestinal disturbances ^a	Rectal incontinence	Impotence	Symptoms of urinary dysfunction		
			Arms	Legs				Loss of sensation of bladder fullness	Delay in initiating voiding stream	Voiding by abdominal strain
A-6	64	8	++	+++	+	0	+	0	0	0
B-3	46	6	++	+++	+++	+	+	+	+	+
B-6	59	8	+	++	+++	+	+	+	+	+
C-2	35	6	++	+++	+++	+	+	0	+	0
F-1	62	6	+	++	+	0	+	0	+	0
J	38	2	+	++	0	0	+	0	+	0
K	57	4	+	++	++	+	0	0	+	0
L	57	7	++	+++	+++	+	+	+	+	+

+ = slight, ++ = moderate, +++ = marked

taining physiological NaCl solution. The other bladder catheter and the stomach catheter were connected to a pressure-recording system consisting of pressure transducers with amplifiers and a multi-channel ink-flow recorder (EMT 34 EMT 31 Mingograph 81 Siemens-Elema, Stockholm). The registration system included subtraction circuit by which continuous subtraction of the intragastric pressure from the intravesical pressure could be obtained and thereby the contribution of straining to the intravesical pressure was deduced. In some cases continuous subtraction was not performed the difference was then obtained by algebraic subtraction on the recorded pressure curves. At micturition the urine was collected in vessel resting on a frame furnished with a strain gauge receptor means of an amplifier and a derivation unit for lining the urine flow this receptor too was connected to the ink-flow recorder. Both the urine volume and the rate of urine flow could be recorded.

The bladder was continuously filled with physiological NaCl solution at a rate of 50 ml/min while the intragastric and intravesical pressures and the difference between these pressures were simultaneously recorded. Every 50th ml instilled saline was noted on the recording. The subject was allowed to void at increasing volumes up to and including that volume at which an urgent desire to void occurred. During micturition the various pressures, the urine volume and urine flow were continuously recorded. In some cases the urine flow was obtained from the recorded volume curve. After each voiding the residual rise was aspirated from the bladder through one of the catheters and was measured.

Definitions

Bladder capacity (ml). The sum of the volume voided and the residual volume on the occasion of micturition.

Table II. Clinical data and findings of urinary bladder function in 8 male controls

Case no	Age (y)	Clinical diagnosis	Bladder capacity ^a (ml)	Residual volume (ml)	Detrusor contraction pressure (mmHg)	Peak flow (ml/sec)	Urethral resistance (mmHg/(ml/sec) ²)
1	53	Hypertonia	420	0	40	9.4	0.82
2	37	Hypertonia	500	0	35	13.9	0.33
3	52	Ulcerative colitis	445	20	33	17.4	0.20
4	43	Pulmonary fibrosis	520	60	26	10.1	0.49
5	42	Cardiomyopathy	350	0	20	24.3	0.08
6	37	Polycythemia	435	0	29	27.0	0.07
7	58	Spicomegaly	370	25	21	9.8	0.51
8	41	Pulmonary sarcoidosis	705	30	26	13.8	0.29
Mean			468	17	29	15.7	0.35
S.D.			112		7	6.7	0.25

^a Volume at urgent desire to void.

between the intravesical pressure when the subject does not strain, and the pre-micturition pressure.

Intrabdominal pressure (mmHg) Intragastric pressure in relation to atmospheric pressure

Peak flow (ml/sec) Maximal urine flow at micturition.

Urethral resistance (mmHg/(ml/sec)²) Intravesical pressure divided by the square of the urine flow

Weak voiding stream	Incontinence	Biopsy confirming amyloidosis
0	0	Skin, rectum
+	+	Skin, rectum
+	+	Skin rectum
+	0	Skin nerve
+	0	Skin rectum
+	0	Skin
+	0	Skin
+	+	Skin, rectum

at which the subject experienced an urgent desire to void.

Intravesical pressure (mmHg). Intravesical pressure in relation to 0-level through the superior border of the symphysis with the subject sitting or standing.

Pre-micturition pressure (mmHg). Intra-cycal pressure immediately prior to micturition—before the pressure rises due to contraction of the detrusor or pelvic floor or due to straining.

Detrusor contraction pressure (mmHg). Difference

RESULTS

Bladder capacity

The bladder capacities registered according to definition are given in Tables II and III. The mean value for bladder capacity in the controls was 468 ± 112 (S.D.) ml. For the patients with amyloidosis the mean value was 664 ± 123 (S.D.) ml. Because of the impaired sensitivity discussed below and because of the histopathological changes previously observed in the bladder wall in this type of patients (3) and the risk of overdistension (13) instillation was interrupted in some patients before an urgent desire to micturate had occurred. In these cases the term "bladder capacity" corresponds to the maximum volume

Table III Findings of urinary bladder function in 8 male patients with primary amyloidosis and polyneuropathy

Case	Bladder capacity (ml)	Volume at desire in void (ml)		Volume at start of micturition/ residual volume (ml)	Detrusor contraction pressure (mmHg)	Peak flow (ml/sec)	Urethral resistance (mmHg/(ml/sec) ²)
		Slight desire	Urgent desire				
A:6	545	N	No	330/170	26	6.4	1.37
B:3	610*	400-500	No	545/225	22	7.9	0.72
				430/165	0	3.7	5.30
				610/ 30	0	14.8	0.22
B:6	750	600-750	N	500/500	0	0	
				700/540	0	5.0	3.36
				750/645	0	3.0	7.56
C:2	700	No	No	340/340	0	0	
				600/525	0	2.0	20.00
				700/560	0	3.0	8.66
F:1	755	500	750	295/120	31	7.6	1.01
				395/ 60	35.5	7.1	1.26
				640/300	21.5	6.8	1.15
				755/215	11	8.0	0.75
J	750	500-750	No	370/370	0	0	
K	435	250	425	750/625	0	5.6	1.91
				150/ 10	0	5.8	0.66
				40/ 65	0	6.9	1.03
L	770	620-770	No	435/ 70	0	6.4	0.43
				665/615	0	4.7	1.78
				770/690	0	11.1	0.39
Mean	664					7.8*	1.06*
S.D.	123					3.7	2.87

* Then incontinent Calculated on the highest, on the lowest individual values

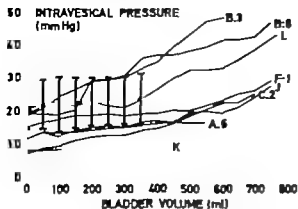


Fig. 1 Intravesical pressure in relation to bladder volume. Observations from 8 controls (mean ± 2 S.D. thick lines) and from 8 patients with amyloidosis (thin lines).

of saline administered and is probably falsely low. Nonetheless 5 patients with amyloidosis had a bladder capacity which exceeded the mean value for the control group by more than $+2$ S.D. The amyloidosis group had a significantly larger bladder volume than the controls ($0.01 > p > 0.001$ according to Student's *t*-test).

As the bladder fills there normally develops a feeling of fullness and the need to micturate. By definition the value for bladder capacity in this study corresponds to the volume at which an nt need to micturate occurred. The volumes high this occurred in the controls are given

in Table II. The mean was 468 ± 112 (S.D.) ml. When the bladder was filled two patients (F.1 and K) experienced an urgent need to void. The volumes at this point were 750 ml and 425 ml respectively. Four patients (B.3, B.6, J and L) had only a slight desire to empty the bladder even at volumes as large as 500, 750, 750 and 770 ml respectively. Two patients reported no desire to void following instillation to 545 ml (A.6) and 700 ml (C.2). They reported only a slight feeling of fullness at these volumes. Four patients (B.6, F.1, J and L) experienced a desire to void urgent or slight only at volumes which exceeded the mean value for the control group by more than $+2$ S.D.

Little or no residual urine was measured in the controls (Table II). In the patients with amyloidosis on the other hand there was a more or less marked tendency to retention (Table III).

Intravesical pressure

The intravesical pressure at various volumes is shown in Fig. 1. In the control group the bladder pressure was initially 20.3 ± 3.2 (S.D.) mmHg. With increasing volume the pressure in this group rose to 31.7 ± 3.9 mmHg at 350 ml (the largest volume which all 8 controls attained). The pressure increase from 0 to 350 ml in this group was 3.4 ± 1.6 mmHg.

The patients with amyloidosis had resting blad

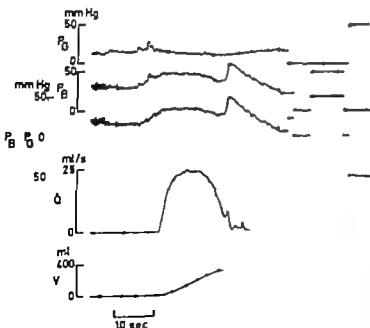


Fig. 2 Simultaneous recordings during micturition without straining from a normal control subject. P_G =intragastric pressure, P_B =intravesical pressure, $P_B - P_G$ =difference between intravesical and intragastric pressure, Q =urine flow rate, V =micturated urine volume.

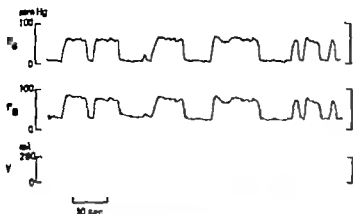


Fig. 3 Recordings during micturition from patient C.2 with insufficiency of the detrusor. Due to straining the intragastric and intravesical pressure curves are almost identical. Abbreviations as in Fig. 2.

der pressures which for small volumes were somewhat lower than the values for the controls. For an empty bladder the pressure was significantly lower in the patients than in the controls ($0.01 > p > 0.001$). The pressure which was 13.7 ± 4.9 (S.D.) mmHg initially increased by 7.1 ± 3.6 mmHg with a volume increase to 350 ml. Patients B.3, B.6 and C.2 had a pressure increase between 0 and 350 ml of 14.5, 17 and 7 mmHg, respectively. These values exceed the mean value for the controls by more than +2 S.D. No patient had a pressure increase that was less than the mean value for the controls -2 S.D. At volumes above 350 ml the intravesical pressure increased further in the patients (Fig. 1).

Detrusor contraction pressure

Values for this pressure which corresponds to the increase of intravesical pressure due to contraction of the detrusor musculature in connection with micturition are given in Tables II and III. All of the controls achieved peak flow during voiding by means of detrusor contraction without contribution from straining (Fig. 2).

All eight patients used abdominal pressure to micturate at the volumes investigated. In six patients (B.3, B.6, C.2, J, K and L) the increase in the intravesical pressure in connection with micturition was completely identical with the increase in intragastric pressure. This is illustrated in Fig. 3. In two patients (A.6 and F.3) the detrusor contraction pressure at peak flow was 26 and 35.5 mmHg, respectively.

Urine flow

The controls had values for maximal flow at micturition, peak flow, between 9.4 and 27.0 ml/sec,

mean 15.7 ± 6.7 (S.D.) (Table II). The peak flow in most of the patients was considerably lower (Table III). The mean value, calculated on the highest individual values was 7.8 ± 3.7 ml/sec. Patient B.3 had a peak flow value of 14.8 ml/sec and patient L a value of 11.1 ml/sec when the bladder volume at the start of micturition was 610 and 770 ml respectively.

Urethral resistance

The resistance to flow in the urethra is shown in Tables II and III. The mean value for the controls was 0.35 ± 0.25 (S.D.) mmHg/(ml/sec)². The mean value for the patients calculated on the lowest individual values was 0.06 ± 0.87 (S.D.). Patient B.3 had a resistance of 0.22 mmHg/(ml/sec)² at an initial bladder volume of 610 ml. Transiently patient L had a resistance of 0.39 mmHg/(ml/sec)² at a large initial bladder volume. At some time all patients had a urethral resistance that exceeded the mean value for the controls by more than +2.5 σ .

Correlation between disturbances of micturition and clinical symptoms

As shown in Table I all the patients except A.6 had experienced difficulties in connection with micturition. Seven reported difficulty initiating micturition. They had also observed weakening of the urine stream. Three patients reported a need to strain in connection with micturition, while the remainder had not noted this. Two patients, A.6 and C., experienced a need to void at bladder volumes of 345 and 700 ml respectively. Prior to the study however they were not aware of any decrease of bladder sensitivity. Patients B.3, B.6 and L reported impairment of the sense of

bladder fullness. At the investigation they reported only a slight need to void at bladder volumes as large as 610, 750 and 770 ml respectively.

At comparison of the grades of other clinical manifestations (Table I) and the degrees of bladder disturbances no consistent agreement was revealed.

DISCUSSION

The fact that most of the patients experienced slight or no desire to void in spite of the presence of a large volume of fluid and a high pressure in the bladder must be regarded as an expression of reduced sensitivity of the bladder wall. The results of this study are in agreement with our previous findings on disturbances of micturition in patients with amyloid neuropathy (3). Histopathological examination of autopsy material revealed deposits of amyloid in the nerves of the bladder wall. Such deposits affecting the nerve fibres can explain the reduced sensitivity demonstrated in the present study.

Normally there are relatively large variations in the resting intravesical pressures between different individuals (12). Nonetheless our patients appeared to have lower pressures at small bladder mes than did the controls.

On the other hand there are only small variations in the resting pressure at different bladder volumes in one and the same person (12). This was the case in all of our controls. Among the patients however the pattern of individual pressure was different. The increase in the intravesical pressure that accompanied increase in the bladder content was strikingly large in most of the patients. This may be attributable to increased rigidity of the bladder wall due to amyloid deposits in the detrusor musculature. Such deposits, which have been observed in autopsy material are described elsewhere (3). Histopathological examination showed that, in place of the smooth muscle fibres in the detrusor were completely replaced by amyloid. This rigidity of the bladder wall is probably analogous to that which occurs in the myocardium in cardiac amyloidosis (14).

In normal subjects the perivesical pressure increases during filling of the bladder (7). Whether the perivesical tissue has an increased rigidity

in these patients with amyloidosis and in what way this may influence the intravesical pressure rise is an open question.

At the time of micturition the intravesical pressure in the controls increased with very little contribution from abdominal pressure. The increase in pressure which occurred due to detrusor contraction agrees with the values measured in other comparable materials (11, 12). All the patients on the other hand needed to strain to micturate at the bladder volumes investigated. In only two of the eight patients could the increase in pressure be partly attributed to detrusor contraction. This impairment of the detrusor function is probably due mainly to amyloid infiltration of the nerves in the bladder. The extent to which amyloid deposits in the musculature decrease the contractility of the detrusor cannot be stated.

Generally the maximal urine flow at micturition was lower and the resistance to flow in the urethra was greater in the patients than in the controls. This can be explained by the impairment of the detrusor function. Normally the detrusor not only brings about an increase in the intravesical pressure but also shortens and widens the proximal part of the urethra (8). The resistance is thus decreased and the urine can pass the "inner urethral orifice". At increased bladder volumes and increased intravesical pressures there was some lowering of the resistance to flow in most of the patients. This can be explained by an increased tension in the bladder wall associated with increased volume leading to some although incomplete widening of the proximal urethra and thereby diminishing urethral resistance. The impairment of the detrusor function is an important factor in the disturbance of the emptying mechanism. Whether alteration in the relaxation capacity of the striated musculature of the urethra plays a role cannot be stated.

The impairment of bladder emptying, demonstrated in these patients with amyloidosis may cause chronic retention and predispose to infection of the urinary tract with risk for pyelonephritis (3).

The disturbance of micturition that occurs in this type of amyloidosis is in some respects similar to that observed in diabetes mellitus with polyneuropathy. In the latter there is also an increased bladder capacity and a tendency to urine

retention (9). Defective opening of the internal meatus of the urethra has been described in connection with roentgenological analysis of bladder emptying and a possible connection with unpaired detrusor function has been discussed (6). Direct measurements of detrusor function and of urine flow however do not seem to have been performed. The bladder in diabetics is reported to be hypotonic compared with that in normals (10-15). In this respect the bladder in amyloidosis with polyneuropathy often seems to differ markedly from that in diabetes mellitus.

ACKNOWLEDGEMENT

This study was supported by grants from the Board for Medical Research of Swedish Life Insurance Companies.

REFERENCES

1. Andersson, R. Hereditary amyloidosis with polyneuropathy. *Acta med. scand.* 183, 85 1970.
2. Andersson, R. & Blom, S. Neurophysiological studies in hereditary amyloidosis with polyneuropathy. *Acta med. scand.* 191 233 1972.
3. Andersson, R. & Hofer, P.-Å. Genitourinary disturbances in familial and sporadic cases of primary amyloidosis with polyneuropathy. *Acta med. scand.* 195, 49 1974.
4. Andrade, C. A peculiar form of peripheral neuropathy. Familial atypical generalized amyloidosis with special involvement of the peripheral nerves. *Brain* 75 408 1952.
5. Araki, S., Mawatari, S., Ohta, M., Nakajima, A. & Kurohwa, Y. Polysyncytic amyloidosis in Japanese family. *Arch. Neurol. (Chic.)* 18 593 1968.
6. Bartley, O., Brodin, I., Fagerberg, S. E. & Wiholm, L. Neurogenic disorders of the bladder in diabetes mellitus. *Acta med. scand.* 180 187 1966.
7. Bjerle, P. Transurethral pressure of the urinary bladder wall. *Acta physiol. scand.* In press 1974.
8. Charkigo, M. Intravesical pressure and outflow resistance during micturition. *Acta neurol. scand. Suppl.* 20: 88 1966.
9. Elienberg, M. Diabetic neurogenic vesical dysfunction. *Arch. Intern. Med.* 117 348 1966.
10. Fagerberg, S. E., Kock, N. O., Petersén, I. & Steiner, I. Urinary bladder disturbances in diabetes. *Scand. J. Urol. Nephrol.* 1 19 1967.
11. Frimodt-Møller, C. & Hald, T. Clinical urodynamics. *Scand. J. Urol. Nephrol. Suppl.* 1 143 1972.
12. von Garretts, B. Intravesical pressure and urinary flow during micturition in normal subjects. *Acta chir. scand.* 114 49 1957.
13. Glahn, H. Overdistension of blæren og principper for kateter- & detsensur regime. *Nord. Med.* 28 128 1971.
14. Gunnar, R. M., Dillon, R. F., Wafiy, R. J. & Elienberg, E. I. The physiological and clinical similarity between primary amyloid of the heart and constrictive pericarditis. *Circulation* 12 827 1955.
15. Kelson, M., Goldberg, P. D. & Mandel, E. H. Neurogenic vesical dysfunction and diabetes mellitus. *N. Y. St. J. Med.* 70 7448 1970.

LUPUS-LIKE SYNDROME ASSOCIATED WITH PULMONARY REACTION TO NITROFURANTOIN

Report of Three Cases

Olof Selroos and Johan Edgren

*From the Fourth Department of Medicine H Isakki University Central Hospital
H Isakki Finland*

Abstract. A systemic lupus erythematosus-like syndrome developed simultaneously with pulmonary reactions of the chronic type in three female patients after treatment with nitrofurantoin for 12, 27 and 38 months, respectively. The syndrome was characterized by elevated ESR, polyclonal hypergammaglobulinaemia, the presence of IgG anti-nuclear antibodies and positive latex-fixation test. Two patients had severe arthralgia and one of them peripheral lymphadenopathy. Pleural effusion and chronic active hepatitis were present in the third patient, in whom interstitial cystitis also developed. All signs and symptoms of the lupus-like syndrome disappeared, without corticosteroid or other medication, when nitrofurantoin was omitted. The dissipation of pulmonary infiltrates and the reversal of interstitial cystitis also appeared to be directly related to cessation of nitrofurantoin therapy. Our findings indicate that long-term medication with nitrofurantoin may cause in addition to pulmonary changes, a simultaneous lupus-like syndrome. Our data also raise the possibility that interstitial cystitis may occur as an adverse reaction to nitrofurantoin therapy.

Since pulmonary reactions to nitrofurantoin were first reported in 1962 (7) an acute and a chronic type of reaction have been documented. The acute reaction, reported with increasing frequency, occurs after readministration of nitrofurantoin to previously sensitized patients. It is regarded as an immunological type III reaction and is characterized clinically by sudden fever, dyspnoea, eosinophilia and pulmonary infiltrates that resemble pneumonia or pulmonary oedema (3, 6, 9). The chronic reaction develops after administration of nitrofurantoin for several months or years and is marked by radiographically demonstrable fibrosis with or without interstitial pneumonitis. Neither fever nor prominent eosinophilia are associated with the chronic reaction. With

the exception of two patients reported by Balk et al (4), no distinct immunological abnormalities have been identified in patients with the chronic reaction (1, 5, 8, 11, 13, 16-21).

We report here on three patients whose reactions to nitrofurantoin were of the chronic pulmonary type and in whom a syndrome typical of systemic lupus erythematosus (SLE) developed simultaneously with the pulmonary manifestations. Interstitial cystitis also developed in one of these patients.

CASE REPORTS

Case 1

Female, born in 1892. Her mother suffered from rheumatoid arthritis. At the age of 40 the patient was afflicted with arthralgia and was, on this account, examined several times during the next ten years. Neither radiographical bone surveys nor serological tests provided any evidence of rheumatoid arthritis.

In March 1969 the patient was admitted to the hospital because of nausea, dyspnoea, dizziness and peripheral oedema. Congestive heart failure was diagnosed and was treated with digitalis and diuretics. Mild diabetes was found and was treated with dietary regimen. Urine culture yielded an abundant growth of *Proteus mirabilis*. Renal function, ESR and peripheral blood picture were normal. An pyelography revealed no abnormalities. Neither rheumatoid factor nor antinuclear antibodies were demonstrable. The Coombs test was negative. Serum protein was 7.6 g/100 ml γ -globulin 1.25 g/100 ml.

Initial treatment of the urinary tract infection with sulphonamide was discontinued after eight days when urticaria developed. On April 14, 1969 treatment with nitrofurantoin, 140 mg daily was started. Four weeks later the patient was discharged in good condition, continuing treatment with nitrofurantoin, digitalis and diuretics. While in hospital her ESR had risen from normal values to 45 and then to 65 mm/h without explanation.

Table 1 Clinical and laboratory data of three female patients with chronic pulmonary reaction and simultaneous lupus-like syndrome induced by nitrofurantoin

Pat. no	Age (y)	Nitrofurantoin therapy		Clinical symptoms and signs	Abnormal laboratory test results	Course after withdrawal of nitrofurantoin without other treatment
		Daily dose (mg)	Duration (mo)			
1	79	150	5	Arthralgia, lymphadenopathy pulmonary infiltrates and fibrosis	ESR 12-1.5 hypergammaglobulinaemia, antinuclear antibodies total titre 1:320 IgG titre 1:320 IgM titre 1:10 latex+ direct Coombs test+ thyroglobulin antibodies titre 1:2.5 mill	Regression of pulmonary infiltrates. Disappearance of the enlargement of lymph nodes and arthralgia. Results of laboratory tests returned to normal
		100	8			
		50	14			
2	61	100	12	Arthralgia, pulmonary infiltrates	ESR 16-80 hypergammaglobulinaemia, antinuclear antibodies total titre 1:1280, IgG titre 1:1280 IgM titre 1:20, latex+	Chest X-ray and results of laboratory tests returned to normal
3	75	150	17	Fever, pulmonary infiltrates and slight fibrosis, pleural effusion, chronic active hepatitis (7)	ESR 23-112, hypergammaglobulinaemia, antinuclear antibodies total titre 1:1000, IgG titre 1:100 IgM titre 1:10 latex++ direct Coombs test+ smooth muscle antibodies+ 1:100	Regression of pulmonary infiltrates. Disappearance of pleural effusion. Improvement in laboratory test results
		100	21			

At follow-up examinations sterile pyuria was repeated finding, but urine cultures yielded no significant bacteriuria. The ESR continued to rise (73-84 mm/h). On 1. 19 1969 the dosage of nitrofurantoin was reduced to mg daily and six weeks later was omitted altogether because of vomiting. The patient's subjective condition proved rapidly after withdrawal of nitrofurantoin and her ESR began to drop (50-37-18 mm/h) the lowest value being recorded four months later. Nitrofurantoin, 100 mg daily was then given again because of pyuria and was continued until Dec. 1971. During this 22-month period the ESR gradually rose from 18 to 125 mm/h serum protein from 8.1 to 9.4 g/100 ml γ -globulin from 2.2 to 3.4 g/100 ml. A chest X-ray taken in Aug. 1970 revealed her lungs to be clear.

When an X-ray taken in Aug. 1971 disclosed patchy pulmonary infiltrates, tuberculosis was suspected. Repeated TB cultures of sputum were negative. The patient was readmitted to the hospital in Nov. 1971 when a follow-up X-ray examination revealed increasing fibrosis of the lungs.

On admission the patient's only complaint was increased pain in various joints. She had no dyspnoea at rest. BP was 160/95. Skin and heart auscultation and thyroid, renal and liver function tests showed normal values. Neither liver nor spleen was palpable but the cervical, axillary and submandibular lymph nodes were enlarged (3-15 mm). ESR was 102 mm/h. The peripheral blood picture was normal except for light eosinophilia (8.5-9.5% of 4700 WBC). Urinary tract examinations revealed a benign papilloma of the bladder that was subsequently electrocoagulated.

Hypergammaglobulinaemia was still present (3.1 g/100 ml) and consisted mainly of an increase in IgG. Immunological tests disclosed the abnormalities listed in Table 1. The Waaler Rose test, however, was negative. LE cells were not found.

Because the pulmonary changes were suspected of being caused by nitrofurantoin, the drug was withdrawn. One month later moderate improvement was already noticeable and since then the chest X-ray has remained unchanged during a follow-up period of 2.5 years. Enlargement of the lymph nodes disappeared within two months of discontinuing nitrofurantoin therapy and the results of laboratory tests have gradually returned to normal values. The ESR fell from 102 to 34 mm/h, the slight eosinophilia disappeared, serum protein and γ -globulin levels returned to normal, the titre of antinuclear antibodies decreased to 1:40 of thyroglobulin antibodies to 1:25 000 erythrocyte antibodies were no longer demonstrable and the latex fixation test is now negative. The patient is currently in fairly good health and is being treated with digitalis and diuretics. The arthralgia persists with the same severity as before administration of nitrofurantoin.

Case 2

Female, born in 1911. Her mother and two sisters suffered from rheumatoid arthritis. The patient was in good health until 1965 when she suffered moderate congestive heart failure. Since 1967 recurrent urinary tract infections have been treated with various drugs. Hypersensitivity to sulphonamides was noted. In March 1971 she was examined in the outpatient department of local hospital

because of acute febrile pyelonephritis. A urine culture yielded growth of *Escherichia coli*. Treatment with nitrofurantol, 100 mg daily was started and subsequently supervised by a local physician. During the follow-up period in 1971 urine cultures showed no bacterial growth. The ESR however gradually rose from normal values to 66 mm/h without explanation.

In Dec. 1971 during visit to the Canary Islands, joint pain, especially in the large joints, developed. No explanation for this pain was found at examination after her return to Finland. Rheumatoid arthritis and infectious arthritis are excluded.

Three months later the patient ESR was 75 mm/h and she was admitted to hospital for further examinations. She was then af- broke and her subjective symptoms were pain in joints and dyspnoea. Physical examination revealed BP of 150/80, a systolic murmur of first degree over the cardiac apex, normal skin and joint state and no enlargement of lymph nodes, spleen or liver. Crepitations rales over the bases of both lungs were however audible and a chest X-ray showed bilateral interstitial infiltrates in the lower lobes of both lungs. The ESR was 80 mm/h blood picture was normal. Urinalysis revealed only slight pyuria. Renal function was normal and renal values were obtained for blood sugar and liver enzyme tests. Serum protein was 8.2 g/100 ml with moderate increase in the γ -globulin fraction (1.8–2.6 g/100 ml). Immunoelectrophoretic investigations showed slight increase in the IgA band. Antinuclear antibodies of the IgG type were demonstrable (Table 1): the latex-fixation test was positive but the Waaler-Rose titre normal. LE cell tests were not done.

Nitrofurantol was suspected of causing the polymorphy changes and the treatment was stopped. Two weeks later moderate regression of the polymorphy infiltrates was observed, and five months later the chest X-ray was normal. Simultaneously the ESR dropped to 25 mm/h, the latex-fixation test was negative, and the titre of antinuclear antibodies decreased to 1/320. One year later (in May 1973) this titre was only 1/20.

The patient is currently in good health. She had neither dyspnoea nor joint pain. Her congestive heart failure is continuously treated with digitalis and diuretics. Urinary sediment and renal function have been normal during the observation period.

Case 3

Female, born in 1899. The patient was in good health until the summer of 1970 when she was troubled with dysuria. A urine culture yielded growth of *E. coli* and she was treated by local physician, first with chloramphenicol and later with nitrofurantol, 150 mg daily. In Nov. 1970 and again two months later the ESR was 44 mm/h and the urinary sediment still showed haematuria, pyuria and Gram-negative bacteria.

In Feb. 1971 she was seen as out-patient in our hospital for further investigation. She was found to be in good health but complained of dysuria and periodic pain over the kidneys. The pulse was 90 and BP 175/105 peripheral blood picture, renal function, chest X-ray and results of heart and lung auscultations were normal. Peripheral lymph nodes, liver and spleen were not palpable. Tests for rheumatoid factor and antinuclear antibodies were nega-

tive. I \bar{I} pyelography revealed morphologically normal kidneys. The ESR had risen to 64 mm/h. The urinary sediment still contained red and white cells in abundance but no bacteria. Urinary TB cultures were negative. Treatment with nitrofurantol was continued.

A cystoscopic examination, performed in June 1971 revealed reddening area near the left ureteral orifice. Tri-bladder volume was 250 ml and biopsy disclosed inflar- urinary changes. When the bladder was emptied on month later gross and microscopical findings were consist- ent with interstitial cystitis.

In Sept. 1971 the urinary sediment still showed haematuria and pyuria. The results of third cystoscopic examination were the same as the earlier tri-bladder volume was 300 ml. In Jan. 1972 biopsy of the bladder still provided the patho-anatomical diagnosis of interstitial cystitis.

In March 1972 the patient had paroxysmal ventricular tachycardia. This episode was promptly corrected with lidocain. Myocardial infarction was excluded. Treatment with digoxin, furosemide and alprenolol was started. The nitrofurantol therapy that had been maintained for 17 months was stopped. Urinary sediment showed slight haematuria but was otherwise normal.

One month later urinalysis revealed red cells in abundance but a urine culture yielded only insignificant growth of enterococci. Nitrofurantol, 100 mg daily as given again as prevention was continued until Jan. 1974. During this period the ESR rose from 30 to 107 mm/h.

In Dec. 1973 the patient fell acutely ill with fever and dyspnoea. When fever persisted for two weeks in spite of treatment with antibiotics she was admitted to the hospital. On admission she was severely fatigued. Nitrofurantol was withdrawn. The temperature was 39.0–39.9°C but returned to normal without antibiotics on the third day in hospital. The pulse rate was 70 respirations 4 and BP 130/70. There were no signs of congestive heart failure. The liver was not enlarged and neither spleen nor peripheral lymph nodes were palpable. The blood picture was normal. Urinalysis and renal function tests showed normal values. Rales and rhonchi were however audible over both lung bases. A chest X-ray showed bilateral infiltrates, most prominently in the middle and lower lobes of the right lung. Pleural effusion was seen on the right side. Serum protein was 7.9 g/100 ml but the γ -globulin fraction was pathologically increased (3.3 g/100 ml). The latex-fixation test was strongly positive but the Waaler-Rose titre only 1/64. Antinuclear antibodies, smooth muscle antibodies and red cell antibodies were also detected (Table 1). The serum bilirubin was normal but five enzyme tests disclosed pathological picture: SGOT was 142 U/l at 25°C SGPT 76 U/l at 25°C, ALDH 315 U/l and alkaline phosphatase 463 U/l (normal below 48 U/l).

Results of liver enzyme tests gradually returned to normal during the 4-month hospital stay. Just before these results were within normal limits, liver scan was taken and found to be normal. A cytological fine-needle aspiration biopsy revealed that hepatocytes were normal but that mononuclear cells, mostly lymphocytes but few plasma cells, were present in increased numbers. Chronic active hepatitis was suspected but histological needle biopsy of the liver was normal.

During the follow-up period, in which the patient was given neither nitrofurantoin nor other antibacterial drugs, a regression of the pulmonary symptoms occurred and the chest X-ray is now almost normal. The ESR has dropped to 40 mm/h. Immunofluorescent antinuclear antibodies are no longer demonstrable, the direct Coombs' test is negative and liver enzyme tests are normal. The latex-fixation test is still positive (+ + 1/64) the Waaler-Rose titre 1/128. The patient has no pain in or swelling of joints, no skin lesions, no enlargement of peripheral lymph nodes. She has no dysuria and a cystoscopic examination in May 1974 revealed quite normal bladder with normal volume (400 ml).

DISCUSSION

Since it was introduced in 1953, nitrofurantoin has been used extensively in the treatment of urinary tract infections. The drug is known to have toxic and allergic side-effects. The most serious toxic side effect is dose-related polyneuropathy, especially prone to occur in patients with impaired renal function treated with normal doses of the drug. Haemolytic anaemia, megaloblastic anaemia, aplastic anaemia and agranulocytosis are rare toxic side effects. The most serious side-effect of the allergic type is an acute pulmonary reaction characterized by sudden fever, dyspnoea and cough. In such reactions pleural effusion, blood eosinophilia and ill-fitrates that resemble pulmonary oedema or pneumonia are common (3, 6, 9).

In 1966 Solliacco et al. (19) drew attention to a case of subacute pulmonary infiltrates caused by nitrofurantoin. Their patient had taken nitrofurantoin daily for one year and symptoms had been present for at least one month. Clearly chronic pulmonary reactions in five patients, the result of treatment with nitrofurantoin, were reported in 1968 by Rosenow et al. (17). Since then similar cases have been documented (1, 5, 8, 11, 13, 16, 18, 20, 21).

The three patients we report on had all been treated for urinary tract infections with nitrofurantoin for long periods—1, 27 and 38 months respectively. Pulmonary infiltrates developed in all three. Partly fibrotic lesions developed in the lungs of two of the patients, of whom one also had pleural effusions. Slight blood eosinophilia was noted in only one patient. After cessation of nitrofurantoin therapy a gradual improvement of the pulmonary manifestations took place without need for simultaneous corticosteroid or other treatment. In all three cases the start of improvement was closely related to the withdrawal of nitrofurantoin, suggesting a direct relationship between the drug and the

pulmonary manifestations. Although histological evidence of the pulmonary lesions we describe is lacking, it seems obvious that these pulmonary reactions are of the same type as those reported previously to have been the result of nitrofurantoin therapy.

Interesting concomitant findings are the immunological abnormalities that developed simultaneously with the pulmonary reactions. These included a markedly elevated ESR, polyclonal hypergammaglobulinaemia, a positive latex-fixation test with either a negative or low Waaler-Rose titre, as well as the presence of IgG antinuclear antibodies. These alterations are typical of SLE, which in 3–12% of cases has been shown to be "drug-activated" (10). The arthralgia of two patients, the peripheral lymphadenopathy of one of them and the possible chronic active hepatitis of the third also conform with the lupus-like syndrome. The findings are very similar to those observed in two patients by Bläck et al. (4).

Toxic hepatic reactions to nitrofurantoin, accompanied by non-haemolytic jaundice, have occasionally been reported (12), but involvement of the liver without jaundice has been documented in only three patients (2, 4). In one of the latter (2) a hepatic reaction with elevated SGOT and alkaline phosphatase levels developed following two weeks' therapy with nitrofurantoin. The patient also had myalgia, arthralgia, an elevated ESR and a diffuse pruritic maculopapular rash on both legs. It seems obvious that the nature of the liver damage without jaundice of this patient is different from that of our case 3, in which hepatic involvement represents not toxic damage but more likely a manifestation of a simultaneously occurring lupus-like syndrome. The infiltration of the liver by mononuclear cells and the presence of smooth muscle antibodies also suggest an immunological rather than a toxic reaction of the liver.

Syndromes indistinguishable from SLE have been reported following the administration of a large variety of drugs. These include hydralazine, procainamide, mephenytoin, diphenylhydantoin, tri-methadione, phenobarbital, isoniazide, penicillin, tetracycline, sulphonamides and others (14). Nitrofurantoin can now be added to drugs causing a lupus-like syndrome.

As has been typical in lupus-like syndromes caused by other drugs, the immunological abnormalities disappeared spontaneously after with-

drawal of nitrofurantoin. The fact that family members of at least two of our patients had a history of other connective tissue disease would support the hypothesis that 'drug-activated' SLE develops only in persons who have a specific predisposition to the disease.

The biopsy-verified interstitial cystitis of our case 3 is also of special interest. It was diagnosed after one year's treatment with nitrofurantoin and was detectable as long as the treatment was continued. Five months after withdrawal of nitrofurantoin a cystoscopic examination revealed a normal urinary bladder. Inasmuch as interstitial cystitis is generally regarded as a chronic disease that terminates in irreversible fibrosis, the course of the illness of this patient has thus so far been unusual. Interstitial cystitis is moreover considered to be an immunological disease (15). Its development therefore in a patient who also develops a lupus-like syndrome secondary to drug therapy is not unexpected and would conform with the postulate of a genetically determined predisposition. However, the possibility that the interstitial cystitis in this case was in fact a reaction to the nitrofurantoin therapy cannot be excluded either, especially in view of the reversibility of the bladder manifestations. The interstitial cystitis may in this patient represent an outward reaction similar to the pulmonary reaction which we believe to be an adverse reaction to nitrofurantoin.

Nitrofurantoin is a widely used and usually well tolerated drug. Although the chronic pulmonary reaction and concomitant lupus-like syndrome that we describe are rare complications, we believe that the calling of attention to such possible adverse reactions might help us to avoid such serious complications.

REFERENCES

1. Barthel E. Nitrofurantoin und Lungenfibrose. *Dtsch. Gesundheits Wes.* 77: 977 1977.
2. Bhagwat, A. G. & Warren, R. E. Hepatic reaction to nitrofurantoin. *Lancet* 2: 1369 1969.
3. Brander L. & Selroos, O. Pulmonary reaction to nitrofurantoin. *Acta med. scand.* 185: 215 1969.
4. Bläck, G. Lindgren, R. & Wiman L.-G. Nitrofurantoin-induced pulmonary fibrosis and lupus syndrome. *Lancet* 1: 930, 1974.
5. David R. H. Andersen H. A. & Stickler G. B. Nitrofurantoin sensitivity: report of child with chronic inflammatory lung disease. *Amer. J. Di. Child.* 116: 418 1968.
6. Editorial. Pulmonary sensitivity to nitrofurantoin. *Brit. med. J.* 4: 704 1969.
7. Israel H. L. & Diamond P. Recurrent pulmonary infiltration and pleural effusion due to nitrofurantoin sensitivity. *New Engl. J. Med.* 266: 1024 1966.
8. Israel K. S. Brubaker R. E. Sharma, H. M. Yuen, M. M. & Glover J. L. Pulmonary fibrosis and nitrofurantoin. *Amer. Rev. resp. Dis.* 108: 353 1973.
9. Larsson, S., Cronberg, S. Dennerberg T. & Ohlsson N. M. Pulmonary reaction to nitrofurantoin. *Scand. J. resp. Dis.* 54: 103 1973.
10. Lee S. E., Rivera, J. & Sargel, M. Activation of systemic lupus erythematosus by drugs. *Arch. intern. Med.* 117: 620, 1966.
11. Lööbbers P. Chronische interstitielle Pneumonie mit Lungenfibrose durch Nitrofurantoin-Langzeittherapie. *Med. Klin.* 66: 818, 1971.
12. Murphy K. J. & Janis, M. D. Hepatic disorder and severe bleeding diathesis following nitrofurantoin ingestion. *J. Amer. med. Ass.* 204: 396 1968.
13. Möller U. Abböhl, K. Blug J. Baumgartner H. Mühlberger F. Scherrer M. & Horgow R. Über empfindlichkeitsreaktionen der Lunge auf Nitrofurantoin. *Schweiz. med. Wochschr.* 100: 2205 1970.
14. Ogryzlo M. A. Systemic lupus erythematosus. I. Rheumatoid diseases (ed. J. J. R. Durbin and W. R. M. Alexander), p. 189. University Press, Edinburgh 1968.
15. Oravisto K. J. Alftam O. S. & Jokinen, E. J. Interstitial cystitis. Clinical and immunological findings. *Scand. J. Urol. Nephrol.* 4: 37 1970.
16. Roosenburg, J. G. Chronische longreactie na gebruik van nitrofurantoin. *Ned. T. Geneesk.* 115: 370 1971.
17. Rosenow E. C. DeRemee R. A. & Dines, B. E. Chronic nitrofurantoin pulmonary reaction. *New Engl. J. Med.* 279: 1258 1968.
18. Runkin, I. Vaisalo, T. & Saarimaa, H. Progressive pulmonary fibrosis during nitrofurantoin therapy. *Scand. J. resp. Dis.* 52: 162 1971.
19. Soffiaco P. A. Robando C. A. & Grace W. J. Subacute pulmonary infiltration due to nitrofurantoin. *Ann. intern. Med.* 111: 1284 1966.
20. Tengelotte P. Chronische Lungenveränderungen nach Nitrofurantoin-Medikation. *Fortschr. Röntgenstr.* 117: 576 1977.
21. Wagner A. Chronische interstitielle Pneumonie mit Lungenfibrose durch Nitrofurantoin-Langzeittherapie. *Med. Klin.* 66: 1808 1971.

COMPLEMENT SYSTEM STUDIES IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

P Teisberg

From Medical Department A, University Hospital and the Institute of Forensic Medicine
Rikshospitalet Oslo Norway

Abstract Complement system involvement has been studied in 16 patients with systemic lupus erythematosus (SLE). Circulating conversion products of C3 were observed in 4 cases. Low mean values of C4 and C3 were found, while C3 proactivator (properdin factor B) levels were low in only a few of the patients. The levels of C4, C3 and C3 proactivator were not lower in the 4 patients in whom C3 conversion products could be demonstrated than in the others. It is concluded that the low complement values found in SLE may be caused mainly by deficient synthesis. Signs of complement activation are in this patient material demonstrated early in the disease, and chiefly in patients not receiving immunosuppressive therapy.

The main manifestations of systemic lupus erythematosus (SLE) are probably caused by circulating complexes of nuclear antigen and antinuclear antibodies (2, 7). Considerable interest has therefore been focused on the chief effector pathway of humoral immunity, the complement system, in SLE. Renal and dermal depositions of C4, C3 and properdin have been taken as evidence of the pathogenetic significance of complement in this disease (16). Profound changes in total haemolytic complement and in levels and activity of individual components of the classic and alternate pathways of complement activation can be demonstrated (4, 5, 6, 8, 11, 13, 14). The finding of low complement activity and low levels of individual factors in SLE has usually been regarded as firm evidence of *in vivo* activation. Studies in recent years have thrown doubts on this concept by showing that low C3 levels in disease could be caused mainly by deficient synthesis of the protein (1). Conflicting results have emerged from turnover studies using radioactively labelled C3, and the pathogenetic significance of the complement system in SLE remains unclear (6, 17).

The present studies were performed with the aim

of elucidating further the mechanism(s) behind the low complement levels often found in SLE.

MATERIAL AND METHODS

Subjects

The patient material includes 16 individuals (11 females). The diagnosis was in each case based on probabed criteria (3). Most patients were studied on one occasion only, six on 2-4 occasions during 2 year period.

Blood samples

Venous blood was collected from both controls and patients and left to coagulate without additives at 20°C. Within 2 hours serum was separated from the blood cell and placed in a freezer at -75°C until the tests were performed 1-10 days later.

Crossed immunoelectrophoresis

Crossed immunoelectrophoresis was performed according to Laurell (9) with slight modifications. The first separation was performed at high voltage (20 V/cm) agarose gel electrophoresis in barbital buffer for 2 1/2 hours as earlier described (18). The gel strip containing the separated serum proteins was cut off and placed in a slot in an anti-C3-containing agarose gel. The second electrophoresis run was then performed at right angles to the first separation and under identical conditions (Fig. 1).

It has been shown by Laurell and Lowén (10), and confirmed in this laboratory, that when the blood samples are handled in the manner described above, and the serum proteins are separated by high voltage electrophoresis on agarose gel, only traces of C3 conversion products can be found in normal serum. In this laboratory 30 serum samples from 35 normal have been tested under these conditions, and no significant amounts of C3 conversion products have been found in any of these sera. EDTA plasma is less reliable in experiments of this kind, possibly because of precipitation of fibrin in the calcium-containing gel (Teisberg, unpublished observations).

Comparison of levels of C3 degradation products can be made between patients, and in one and the same patient on different occasions by planimetry. In this study the

Converted

C3

C3

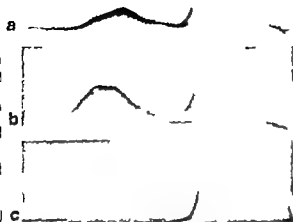


Fig. 1 Crossed immunoelectrophoresis of sera from SLE patients with circulating conversion products of C3 (a, b) and normal control serum examined by the same method (c). Anode is to the left in the first electrophoretic separation and on the top in the second.

sera have mainly been classified as positive or negative with regard to content of C3 conversion products.

Immunokhemical determinations

C4, C3 and C3 proactivator (properdin factor B) determinations were performed by the radial immunodiffusion method according to Mancini et al. (12)

Antisera

Anti-C3 was produced in rabbits immunized with human C3 isolated by preparative electrophoresis on agarose gel. The antiserum contained two weak contaminating antibodies. The antiserum used in radial immunodiffusion experiments was therefore absorbed with a patient serum deficient in C3. Specific anti-C4 was provided by Dr C. Alper, Boston. Anti-C3 proactivator was obtained from Behringwerke, Marburg/Lahn.

Reference serum

Standard human serum (Behringwerke) was used as reference serum for the determination of C3 and C4 in the radial immunodiffusion experiments.

RESULTS

Relevant clinical data concerning the patient material are given in Table I.

C3 conversion products were demonstrated on at least one occasion in 4 of the 16 patients with SLE

(Table II). Of the 6 patients tested on several occasions 5 were consistently negative. One patient (no. 8) had initially circulating conversion products of C3. Later on, after initiation of prednisone and azathioprine treatment, she became negative and has remained negative and has been in complete clinical remission for one year. Three of the 4 positive patients did not receive immunosuppressive therapy at the time of the study, while 9 of the 12 negative patients were on prednisone alone or combined with azathioprine.

C4, C3 and C3 proactivator levels are given in Table II. It can be seen that mean levels of both C4 and C3 were lower than normal mean. Only two patients had C4 levels and one patient had a C3 level above the normal mean. In 4 patients extremely low levels of both components were found. There is good correlation between C4 and C3 levels in the individual patients ($r=0.9049$, $p<0.001$). The mean C3 proactivator level in the patient group did not differ significantly from that found in normals. The patients with very low C4 and C3 levels, however, had also lower than normal C3 proactivator levels.

No differences in complement component levels were found between the groups with and without C3 conversion products.

Disease activity as judged by clinical symptoms and signs was present in all 4 patients with C3 conversion products, but also in 5 of the other 12 patients. Lower mean levels of C4 and C3 were found in the patients with disease activity than in those with non-active SLE, but the differences were not statistically significant.

DISCUSSION

It is generally accepted that total haemolytic complement, biological activity of several of the factors and the levels of these factors measured immunokhemically are often decreased in SLE patients. In this study low C4 and C3 levels have been found, and the decrease is of approximately the same degree for both factors. Low C3 proactivator levels were found less constantly. Others have reported that low C4 levels may be seen even more often than low C3 levels (8, 15). C1q and C2 are usually depressed while one study reports normal C5 levels (8). Properdin levels are low in patients with high disease activity (13). This shows that the complement abnormalities in this disease affect both the classic and the alternate or properdin pathways.

Table 1 Clinical and laboratory data

For patients studied more than once the first set of laboratory values is given

Case no	Sex	Duration		ESR	ANF	LE cells	IgG	IgM	IgA	Treatment when first seen	Organ manifestation
		Age (y)	(y)								
1	♀	23	10	6	+	+	1 200	130	70	Prednisone	Nephritis mucocutaneous lesions, serositis
2	♀	33	13	26	+	+	2 750	80	40	Prednisone azathioprine	Nephritis arthritis, mucocutaneous lesions
3	♀	III	4	11	+	+	2 050	140	300	None	Arthritis, vasculitis, lymphadenopathy
4	♀	34	3	18	+	+	1 800	120	180	Prednisone, azathioprine	Nephritis, arthritis, pancytopenia
5	♀	26	6	29	+	+	1 300	110	310	None	Nephritis, arthritis, pancytopenia, mucocutaneous lesions
6	♀	33	13	85	+	+	2 300	200	190	Prednisone azathioprine	Nephritis, vasculitis, mucocutaneous lesions
7	♀	26	6	11	+	+	150	140	60	Prednisone	Nephritis vasculitis, mucocutaneous lesions, CNS manifestations
8	♀	28	2/12	31	+	+	2 000	620	220	None	Nephritis, arthritis, mucocutaneous lesions
9	♀	27	4	11	+	-	1 650	50	40	Prednisone azathioprine	Arthritis, mucocutaneous lesions, CNS manifestations
10	♀	44	6	72	+	+	100	200	180	Prednisone	Nephritis arthritis, mucocutaneous lesions
11	♂	34	5	105	+	+	1 800	190	670	Prednisone	Vasculitis mucocutaneous lesions, CNS manifestations
12	♀	32	10	60	+	+	2 650	190	250	Prednisone, azathioprine	Nephritis, arthritis, mucocutaneous lesions, thyroiditis, liver manifestations
13	♂	38	4	13	+	+	2 750	90	70	None	Nephritis, CNS manifestations, liver manifestations
14	♂	34	6	30	-	+	2 500	340	530	None	Arthritis, mucocutaneous lesions, vasculitis
15	♂	29	1	41	+	-	2 200	280	320	Prednisone	Nephritis, arthritis, vasculitis
III	♂	52	1	105	+	+	3 350	120	140	None	Nephritis, arthritis mucocutaneous lesions, vasculitis, carditis

Alper and Rosen (1) were the first to show by turnover studies of radioactively labelled C3 that low levels of this factor in glomerulonephritis could be caused by decreased synthesis and not necessarily by *in vivo* activation. Humsicker et al (6) in their study of a large group of SLE patients concluded that the low C3 levels were caused mainly by complement activation. In contradiction to this the turnover study of Sliwinski and Zwaifler (17) showed that decreased synthesis was the chief reason for the low C3 content in their SLE patient sera.

In the present patient group *in vivo* activation of C3 seems to take place at a significant rate in only a small proportion. Even the sera containing C3 degradation products contain rather low levels compared to what can be found for instance in some patients with glomerulonephritis and chronic active hepatitis (19, 20) and some of these latter patients may even have normal C3 levels. One of the present patients activating C3 had a C3 level at the normal mean and of the three patients with very low C4 and

C3 levels (nos 2, 15, 16) C3 activation could be demonstrated in only one. The present results thus support the findings reported by Sliwinski and Zwaifler (17) and it may be concluded that in this group of patients low complement factor levels cannot be explained by *in vivo* activation alone. It is possible, however, that the results reported by Humsicker et al (6) are caused by the selection of patients in an early stage of the disease.

In the present patient material a moderate degree of *in vivo* activation of C3 is found in some individuals generally said in those with relatively short disease duration and those who have not been treated with immunosuppressive drugs. It seems that the immunological activity turns out to some extent after a few years, even if the disease process propagates in some organ system. The seemingly low activity in the humoral immune system may also be the result of intense immunosuppressive therapy. In some patients as apparently in the case in our patient 8 it may be concluded that in the present

Table II Results of the screening for circulating conversion products of C3 and of the immunochemical determinations of C4, C3 and C3 proactivator levels in serum

The evaluation of disease activity is based on clinical symptoms and signs. For patients studied more than once the first set of results is given

Case no.	Disease activity	C3 conversion products	C4 (mg/100 ml)	C3 (mg/100 ml)	C3 proactivator (% of normal mean)
1	-	-	18.8	57	105
2	+	-	0.8	13	60
3	-	-	18.8	55	99
4	-	-	19.0	56	104
5	-	-	13.1	38	82
6	+	-	17.4	45	74
7	+	-	19.8	46	100
8	+	+	16.2	36	104
9	+	+	21.2	49	111
10	-	-	22.0	54	111
11	+	-	25.8	60	114
12	-	-	14.6	38	73
13	+	+	24.3	85	136
14	-	-	26.3	98	158
15	+	-	0.9	11	65
16	+	+	1.1	9	42
Mean for patient			16.3	46.9	96.2
Mean for normals			23.4	97.0	100
Normal range (mean \pm 2 S.D.)			12.8-34.0	64-130	68-132

ent group the possible pathogenetic significance complement activation is mainly limited to the early phase of the disease

The reason for the low complement factor levels found in so many SLE patients remains unknown. A negative feed-back system, which acts to protect the body from the effects of complement activation by depressing the synthesis has been proposed (17) but represents at present only a theory

REFERENCES

- Alper C. A. & Rosen, F. S. Studies of the in vivo behavior of human C3 in normal subjects and patients. *J. clin. Invest.* 46: 2021 1967
- Christian, C. L. Immuno-complex disease. *New Engl. J. Med.* 280: 878 1969
- Cohen, A. S., Reynolds, W. E., Franklin, E. C., Rifkin, J. P., Ropes, M. W., Schulman, L. E. & Wallace, S. L. Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. rheum. Dis.* 21: 643 1971
- Gewurz, H., McKering, R. J., Mergenhagen, S. E. & Good, R. A. The complement profile in acute glomerulonephritis, systemic lupus erythematosus and hypocomplementemic chronic glomerulonephritis. *Int. Arch. Allergy* 34: 556, 1968
- Gotloff, S. P., Isaacs, E. W., Mechrcke, R. C. & Smith, R. H. Serum beta₂-globulin in glomerulonephritis and systemic lupus erythematosus. *Ann. Intern. Med.* 71: 327 1969
- Hunticker, L. G., Ruddy, S., Carpenter, C. B., Schur, P. H., Merrill, J. P., Möller-Eberhard, H. J. & Aueren, K. P. Metabolism of third complement component (C3) in nephritis. *New Engl. J. Med.* 287: 835 1972
- Koffler, D., Agnello, V., Winchester, R. & Kunkel, H. G. The occurrence of single-stranded DNA in the serum of patients with systemic lupus erythematosus and other diseases. *J. clin. Invest.* 52: 198 1973
- Köhler, P. F. & Ten Brugel, R. Serial complement component alterations in acute glomerulonephritis and systemic lupus erythematosus. *Clin. exp. Immunol.* 4: 191 1969
- Laurell, C. B. Antigen-antibody crossed electrophoresis. *Ann. Biochem.* 10: 358, 1965
- Laurell, C. B. & Laurell, B. Electrophoretic studies of the conversion products of serum beta₂-globulin. *Immunology* 12: 313 1967
- Lewis, E. J., Carpenter, C. B. & Schur, P. H. Serum complement component levels in human glomerulonephritis. *Ann. intern. Med.* 75: 555 1971
- Mancini, G., Carbonara, A. O. & Heremans, J. F. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochimistry* 2: 235 1965
- McLean, R. H. & Michael, A. F. Properdin and C3 proactivator: Alternate pathway components in human glomerulonephritis. *J. clin. Invest.* 52: 634 1973
- Morse, J. H., Möller-Eberhard, H. J. & Kunkel, H. G. Antinuclear factors and serum complement in systemic lupus erythematosus. *Bull. N. Y. Acad. Med.* 38: 641 1962
- Petz, L. D., Sharp, G. C., Cooper, N. R. & Ivins, W. S. Serum and cerebral spinal fluid complement and serum autoantibodies in systemic lupus erythematosus. *Medicine* 50: 259 1971
- Rothfield, N., Ross, H. A., Mitsu, J. O. & Lepow, I. H. Glomerular and dermal deposition of properdin in systemic lupus erythematosus. *New Engl. J. Med.* 287: 681 1972
- Schwartz, A. J. & Zwafler, N. J. Decreased synthesis of the third component of complement (C3) in hypocomplementemic systemic lupus erythematosus. *Clin. exp. Immunol.* 11: 21 1972
- Telsberg, P. High voltage agarose gel electrophoresis in the study of C3 polymorphism. *Vox Sang.* 19: 47 1970
- Telsberg, P. & Gjone, E. C. Utilizing conversion products of C3 in liver disease. Evidence for in vivo activation of the complement system. *Clin. exp. Immunol.* 14: 509 1973
- Telsberg, P., Grottnum, K., Myhre, E. & Flatzmark, A. In-vivo activation of complement in hereditary nephropathy. *Lancet* 2: 356, 1973

FOLLOW-UP

What happened to the patients?

Readers of scientific medical publications often complain about the fact that the paper is produced very soon after the patient has been observed and treated. This means that we do not get an overview of the whole disease but often just a glimpse of one particular stage.

The Editors have decided to introduce a special

section in the Acta Medica Scandinavica under the heading FOLLOW UP WHAT HAPPENED TO THE PATIENTS?

Our authors are invited to give further information about the development of interesting cases whose history has been published previously. The first report in this series is presented below.

ACUTE LEUKEMIA AND ACHRESTIC ANEMIA IN A BROTHER AND SISTER

Jørgen Bichel

From the Institute of Cancer Research, Radium Centre, Aarhus, Denmark

In 1940 the author published an article entitled Acute leukemia and achrestic anemia in brother and sister (*Acta med. scand.* 104: 578 1940). The sister died of acute myeloid leukemia. The brother who suffered from a refractory megaloblastic-macroblastic anemia has later de-

veloped an acute leukemia so that the cases correctly should be classified as familial leukemias.

Sponsored by the Danish Cancer Society

BOOK REVIEW

Third Conference on Cooley's Anemia The New York Academy of Sciences, Vol 232, 380 pages \$33.00 New York 1974

Thalassemia—in this volume called Cooley's anemia—is a disease with a characteristic geographic pattern of distribution. As a matter of fact Cooley's anemia is a misleading title of a book treating both the heterozygous and the homozygous states of the many different thalassemias. Cooley's anemia is still sometimes used as an eponym for the much rarer homozygous condition.

Until recently even highly specialized hematologists have had very little personal experience from these interesting diseases if they work in Northern Europe. During the last decade the gigantic migrations of foreign labour have brought people of Mediterranean origin to our latitudes. The present number of this journal contains descriptions

Norwegian patients who may be examples of novel mutations leading to deficient synthesis either the α or the β chains in the globin molecule also in our part of the world.

As a biological problem thalassemia has long been of great interest both to protein chemistry, molecular medicine and genetics as well as to clinical hematology and geographical pathology. This volume is the third in a series of conferences on the disease. It contains valuable information and stimulating discussion by leading specialists within the field and gives excellent illustrations of the fact that clinical medicine on the molecular level is a reality for millions of people.

Already in the opening remarks the Chairman

discusses the topical problem genetic engineering when he asks the question how to bypass the genetic defect. Different specialists on protein biosynthesis discuss the possible mechanisms for defective synthesis of certain polypeptide chains in this group of diseases. It seems as if the function of messenger RNA were disturbed. Many of these papers seem to be far away from routine medicine even if they discuss fundamental problems in the etiology of common disease. There is however also discussion of such clinical problems as the dangers (iron overload) or advantages of massive transfusions, the nature of urinary pigments in these diseases, interaction between different thalassemic defects and other genetically determined hemoglobinopathies or enzyme deficiencies and the question of intrauterine diagnosis.

The chapter by Quattrin and Venturolo tells the story of a disease studied intensely in Naples and Southern Italy ever since it was first found in an Italian patient some 15 years ago. This so-called Lepore hemoglobin is an experiment of nature that has taught as much regarding the interaction between different genes that determine the synthesis of globin polypeptides. The almost innumerable hemoglobinopathies are experiments by nature discovered at the bedside that cannot be repeated in the laboratory.

The volume may be recommended to clinicians with an interest in fundamental medicine and—of course—to the biologist working in the basic sciences.

Jan G. Waldenström

MYOCARDIAL BIOPSY IN A CASE OF CARDIOMYOPATHY AND PARTIAL α -1 ANTITRYPSIN DEFICIENCY WITH LIVER ENGAGEMENT

Ålf Torp

From the Heart Laboratory Department of Medicine, University of Lund, Malmö General Hospital, Malmö, Sweden

Abstract. A case of partial α -1 antitrypsin deficiency with liver engagement and biopsy-verified congestive cardiomyopathy is reported. The possibility of a connection between these two diseases is discussed in view of recent literature.

Since the genetic deficiency of α -1 antitrypsin and its association with emphysema was described by Laurell and Eriksson (12) increasing interest has been directed towards the significance of deficiency of this glycoprotein (11). Present in normal concentration in human serum it is a powerful inhibitor of a variety of proteolytic enzymes. Deficiency states may be homo- or heterozygous with a serum concentration of α -1 antitrypsin within the ranges of 5-85% of normal. The incidence of homo- or heterozygous deficiency is estimated at approximately 1/30 in a normal population (7, 8). The different deficiency states are classified according to the immunological phenotype in the Pi (protease inhibitor) system (8) where the homozygous deficiency is designated for instance PP^{00} and the heterozygous PP^{01} .

Whereas the first reports described panlobular emphysema associated with homozygous α -1 antitrypsin deficiency it has later been shown that persons with partial deficiency are also prone to develop lung disease as well as cirrhosis (4, 5). One case with α -1 antitrypsin deficiency in association with emphysema, angitis and glomerulonephritis has been described (16).

Recently we had the opportunity to investigate a patient with heterozygous α -1 antitrypsin deficiency with liver engagement and a congestive cardiomyopathy which gave rise to the question whether there might be a connection between

these two diseases and the protease inhibitor deficiency.

CASE REPORT

A man, born in 1974, who apart from cholecystectomy in 1962 has been in good health and working as a school porter. In spring 1977 increasing fatigue, dyspnea and ankle edema. The patient was considered to have developed heart failure and digitalis and diuretics were instituted with good but temporary effect. Five months later the symptoms returned despite continuous treatment. The patient now seemed to be more seriously ill and was admitted to our department for cardiological examination. Upon admission in Jan 1978 he showed signs of severe right heart failure with congested neck veins in the sitting position, gross liver enlargement and ankle edema. He complained of fatigue, dyspnea as well as transient dizziness.

Physical examination gave no evidence of valvular heart disease. BP 135/95, chest X-ray which some months earlier had shown a moderate but uncharacteristic heart enlargement was now normal. On several occasions the ECG showed sinus rhythm with slight ST-T changes corresponding to the left ventricle. These changes could, however be attributed to the digitalis treatment. A bicycle ergometer test, starting with 4 min at 300 kpm/min, was discontinued at 600 kpm for 2 min because of dyspnea. During and after the test, certain acceleration of the ST-T changes was noted. Spirometry was performed with a Bernsteins spirometer and normal values according to Björklund et al. (2) were applied. The functional residual capacity was measured by nitrogen wash-out method (3). The vital capacity and total lung capacity corresponded to 65% of predicted values and FEV 1.0% of vital capacity was 74. Consequently the patient had pronounced restrictive lung disease.

Routine laboratory findings were normal except for elevated unconjugated bilirubin and spontaneously lowered prothrombin index to about 50% of normal. The hepatomegaly was further investigated by L. infusion of Tc 99 m-sulphur colloid showing moderately enlarged liver and spleen. Liver biopsy showed histo-

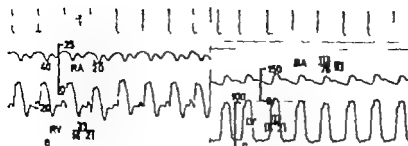


Fig. 1 ECG and pressure tracings from right and left heart catheterization in Jan 1973. RA=right atrium, RV=right ventricle, LV=left ventricle, BA=brachial artery (mmHg). Upper tracing: ECG lead V₄. Paper speed 25 mm/sec.

chemical changes, consistent with α -1 antitrypsin deficiency with the typical rounded globules within the hepatocyte (4, 18) which were resistant to treatment with diastase. A routine electrophoresis was normal with an α -1 antitrypsin concentration of 80 mg/100 ml. A double crossed immunoelectrophoresis according to Laurell (11) was done, revealing that the patient had a partial α -1 antitrypsin deficiency of Pi type MZ.

Right and left heart catheterization was performed. The patient had pathologically elevated end-diastolic pressures in the right as well as the left ventricle and grossly elevated pressure in the right atrium (Fig. 1).

Permanent values from this investigation and from another catheterization made 10 months later are given in Table 1. Angiocardiography showed normal pulsations in all heart chambers, normal valves and no signs of constrictive pericarditis. The pulmonary artery showed normal anatomy without signs of basal hypoplasia.

Semi-selective coronary arteriography revealed normal arteries and normal aortic valves. In con-

nection with the catheterizations, endomyocardial biopsies according to Kono and Sakakibara (10) were done. On two occasions the sections from the right ventricle showed a rather widespread fibrosis and signs of hypertrophy (Fig. 2).

Large amounts of PAS-positive material occurred in and between the myofibrils. This material was glycogen since it disappeared upon treatment with diastase. In some diastase-treated sections, however, it was possible to recognize some small PAS-positive granules (Fig. 3). The appearance of these granules was not like those from this patient's liver and they were considered to be lysosomes. To exclude abnormalities in glycogen metabolism, biopsy from skeletal muscle was analysed and no such abnormality could be demonstrated.

The investigation thus ended in a case showing heart failure. Biochemical changes in the liver consistent with antitrypsin deficiency and a restrictive lung disease. About one year later the spirometry was completed with a full pulmonary investigation including regional lung function measured by ¹³³Xenon radionuclide spirometry (17). The values for perfusion per lung volume showed normal perfusion gradient between apices and bases in the sitting position. The typical picture of emphysema in association with α -1 antitrypsin deficiency implies an altered distribution of pulmonary perfusion with loss of this gradient. This method is considered the most sensitive for detection of early involvement of the pulmonary vascular bed (13).

The patient has been treated orally with digitalis, diuretics and spironolactone since the first investigation. In addition he has received 20 mg furosemide and 200 mg theophylline s. twice a week.

DISCUSSION

The connection between α -1 antitrypsin deficiency and emphysema has been known for more than a decade (16): cirrhosis of the liver has also been described in this protein abnormality (19), and as mentioned a single case with angitis and glomerulonephritis (16). Recently Campa et al.

Table 1 Values from the two catheterizations

RA=right atrium, RV=right ventricle PA=pulmonary artery PCV=pulmonary artery wedge LV=left ventricle BA=brachial artery (pressures in mmHg) HR=heart rate (beats/min) CO=cardiac output (l/min) SV=stroke volume (ml/beat)

	Jan. 1973	Oct. 1973
RA	20	13
RV	34	28
PA	16-21	10-16
	28-22	28-19
	20	15
PCV	19	19
LV	113	89
	16-21	11-15
BA	115-92	89-72
	78	61
HR	84	86
CO	3.86	4.59
SV	46	53



Fig 2 Biopsy from the right ventricle showing widespread interstitial fibrosis and signs of hypertrophy of muscle fibres. Hematoxylin-eosin, $\times 116$.



Fig 3 Biopsy from the right ventricle diastase-treated PAS staining. Arrows indicate PAS-positive granules. 291

(4) reported a case of cirrhosis associated with partial α -1 antitrypsin deficiency. As far as we know antitrypsin deficiency has up to now not been reported in connection with primary myocardial disease. The case presented here fulfils the criteria of a congestive cardiomyopathy (9) the etiology of which according to the definition is unknown.

The pathogenic mechanism of the development of lung and liver disease associated with antitrypsin deficiency is not clearly understood. Two separate theories have been put forward. According to Eriksson (6, 7) the antienzyme deficiency renders the tissue more vulnerable to damage by the lack of defence against liberated proteases. Lieberman et al. (15) consider the position of intracellular antitrypsin to be of importance in developing the liver damage. Lieberman (14) has also demonstrated the presence of degraded antitrypsin in the sera of patients with homo- or heterozygous antitrypsin deficiency. This degraded antitrypsin has abnormal antigenicity which may give rise to antigen-antibody reactions causing tissue injury. Whatever the mechanism may be several authors have stressed the importance of the damage of elastic tissue. As the hemodynamic investigations in this case have given values corresponding to loss of elasticity in the left as well as the right ventricle, it might be postulated that the supportive tissue of the heart has been damaged through lack of the inhibitory protein. It seems improbable that α -1 antitrypsin de-

ficency alone can give rise to tissue damage since even individuals with homozygous deficiency have been protected from developing either the liver or the lung disease (18). It is certainly more likely that other unfavourable agents in inhibitor deficiency can initiate a chain of events leading to decreased function of an organ which is dependent upon its connective tissue. This theory can be attributed to the heart as well as the lung provided that the myocardium has been exposed to for instance a myocardium increased work load as in hypertension or toxic influence such as alcohol. These factors could have been present without clinical manifestations at the initial stage but nevertheless may have caused irreversible changes in the myocardium. As the patient did not show any signs of emphysema of the lung his heart failure cannot be secondary to this disease.

It is difficult to draw significant conclusions from a single case. The combination of partial α -1 antitrypsin deficiency with pathognomonic changes in the liver and widespread fibrosis demonstrated in repeated biopsies from a severely damaged myocardium in this case of congestive cardiomyopathy gives rise to an hypothesis concerning its etiology. α -1 antitrypsin has well documented protective roles in inhibiting the action of certain enzymes such as trypsin, collagenase, elastase and leucocyte proteases. Severe or intermediate deficiency of α -1 antitrypsin may render the myocardium more vulnerable to noxious factors.

which in the presence of normal antitrypsin concentration would not lead to any clinical consequences

ACKNOWLEDGEMENT

This work was supported by grants from the Swedish National Association against Heart and Chest Diseases and the Hilda Almqvist Foundation.

REFERENCES

- 1 Alper C. A. Deficiency of alpha-1 antitrypsin. *Ann. intern. Med.* 78: 298, 1973
- 2 Berglund E., Birath, G., Bjure, J., Glimby G., Kjellmer I., Sandqvist L. & Söderholm, M. Spirometric studies in normal subjects. I. Forced expirations in subjects between 7 and 70 years of age. *Acta med. scand.* 173: 185, 1963
- 3 Bouhuys, A., Hagstam K. E. & Lundin, G. Efficiency of pulmonary ventilation during rest and light exercise. A study of alveolar nitrogen wash-out curves in normal subjects. *Acta physiol. scand.* 35: 289, 1956
- 4 Campa, J. L., Craig, J. R., Peters, R. L. & Reynolds T. B. Cirrhosis associated with partial deficiency of alpha-1 antitrypsin in an adult. *Ann. intern. Med.* 78: 233, 1973
- 5 Cohen K. L., Rubin, P. E., Echevarria, R. A., Sharp, H. L. & Teague P. O. Alpha-1 antitrypsin deficiency emphysema and cirrhosis in an adult. *Ann. intern. Med.* 78: 227, 1973
- 6 Eriksson S. Pulmonary emphysema and alpha-1 antitrypsin deficiency. *Acta med. scand.* 175: 197, 1964
- 7 -- Studies in alpha-1 antitrypsin deficiency. *Acta med. scand. Suppl.* 432, 1965
- 8 Fagerhol, M. The Pi-system. Genetic variants of serum alpha-1 antitrypsin. *Ser. Haemat.* 1: 153, 1968
- 9 Goodwin, J. F. & Oakley C. M. The cardiomyopathies. *Brit. Heart J.* 34: 545, 1972
- 10 Konno, S. & Sakakibara, S. Endomyocardial biopsy. *Dis. Chest* 44: 345, 1963
- 11 Laurell C.-B. Antigen-antibody crossed electrophoresis. *Analyt. Biochem.* 10: 358, 1965
- 12 Laurell C.-B. & Eriksson S. The electrophoretic alpha 1 globulin pattern of serum in alpha-1 antitrypsin deficiency. *Scand. J. clin. Lab. Invest.* 13: 132, 1963
- 13 Levine H. W., Thalamo, R. C., Shannon, B. C. & Kazem, H. Alteration in distribution of pulmonary blood flow. An early manifestation of alpha-1 antitrypsin deficiency. *Ann. intern. Med.* 73: 397, 1970
- 14 Lieberman J. A new "double ring" screening test for alpha-1 antitrypsin variants. *Amer. Rev. Resp. Dis.* 108: 248, 1973
- 15 Lieberman J., Mittman, C. & Gordon H. W. Alpha-1 antitrypsin in the livers of patients with emphysema. *Science* 175: 63, 1972
- 16 Miller F. & Kaschner M. Alpha-1 antitrypsin deficiency emphysema, necrotizing angitis and glomerulonephritis. *Amer. J. Med.* 46: 615, 1969
- 17 Milner G. ¹³³Xe-radonspirometry. A clinical method for studying regional lung function. *Scand. J. Resp. Dis. Suppl.* 64, 1968
- 18 Sharp, H. L. Alpha-1 antitrypsin deficiency. *Hosp. Practice* 5: 83, 1971
- 19 Sharp H. L., Bridges, R. A., Krivit W. & Freier E. F. Cirrhosis associated with alpha-1 antitrypsin deficiency: A previously unrecognized inherited disorder. *J. Lab. clin. Med.* 73: 934, 1969

ERYTHRODERMIA SÉZÁRY WITH IMMUNOLOGICAL DEFICIENCY AND ANTIBODIES AGAINST HUMAN ALBUMIN

Valentin Iliescu, Leif Lindholm and Annela Ehreström

From the Medical Department, Lindsberg Hospital, Lindsberg, the Institute of Medical Microbiology, Göteborg, and the Department of Immunology, the National Bacteriological Laboratory, Stockholm, Sweden

Abstract A case of erythrodermia Sézary in which lymphocyte stimulation tests and quantitation of T and B lymphocytes were performed, is reported. The patient developed during 10 years of evolution an immunological deficiency with low IgA, IgG and IgM values. Passive hemagglutination techniques showed an antibody titer of 1:8000 against human serum albumin.

Sézary and Bouvraïn (17) described in 1938 a syndrome characterized by erythrodermia, pruritus, leonine facies. They found in the peripheral blood atypical mononuclear cells which they believed belonged to the histiocytes. Baccarella (1) described in 1939 a somewhat similar entity but claimed a "lymphomonocytic" origin of the circulating cells. Following the original description a number of cases or series have been published all over the world and various interesting points have been reported. We have had the opportunity to follow a patient who presented with the classical clinical and cytological features of the Sézary syndrome and besides slowly developed an immunological deficiency.

METHODS

Routine hematological techniques have been used for the examination of the blood and bone marrow. Duplicate samples were sent for cross-examination to the Centre d'Hématologie de Bordeaux, France (Prof. J. Moulonnier). Quantitative immunoelectrophoresis determinations were performed at Malmö General Hospital (Prof. C. B. Lönnell).

In vitro function tests and quantitation of T and B lymphocytes are performed. For these tests heparinized blood was diluted 8 times in Eagle's medium containing 10% calf serum. Streptococci and penicillin were added. Portions of the diluted blood (0.2 ml) were cultured in the wells of Falcon Microtiter II plates. Optimal amounts of mitogen or antigens are added. After 5 days incubation at 37°C 2 μ Ci ³H-thymidine (5 Ci/mmol) from the Radio-

chemical Centre, Amersham, England, was added. Eighteen hours after the addition of ³H-thymidine the cells were washed on millipore filters. The filters were then dried and dissolved in Solben (Packard Instrument Co.) and the radioactivity was determined in a Packard Ser 3000 Liquid Scintillation Spectrophotometer using Permablend® (Packard) as toluene. The quantitation of T and B cells was performed according to the rosette formation test as described by Jondal et al. (12). Demonstration of surface immunoglobulins was performed by indirect immunofluorescence on cells in suspension by the sequential addition of rabbit antimouse FAB (Behringwerke, Marburg, Germany) and antirabbit immunoglobulin labeled with FITC (SBL, Stockholm, Sweden). The mitogens used were: phytohemagglutinin (PHA) (Difco Lab., Detroit), concanavalin A (Kiles-Yedda Ltd., Israel) and pokeweed mitogen (Grand Islands Biol. Co., N.Y.). Antigens used were: PPD (Statens Serum Institut, Copenhagen), Momba albumin (Hoffman-La Roche, S. Pauline, Wisc.), Yersinia® (Lederle Lab.) and Trychothryon Extr. (N. V. Hall Aalberg Lab., Harlem). Passive agglutination against bovine serum albumin (BSA) (hydrolyzed, Hopkin & Williams) and human serum albumin (HSA) (hydrolyzed, Kabi, Sweden) was performed according to Boyden (*). Sheep red blood cells were coated with BSA and HSA respectively.

CASE REPORT

The patient, 63-year-old woman, was first hospitalized in 1964 for "fever and chills". The only clinical finding was a macromor over art. temporale. No palpable lymph nodes were found. The laboratory findings were: ESR 71 mm/h, WBC 31000/mm³ and normal differential count. Bone marrow puncture showed no abnormalities. Serum electrophoresis showed "high α_2 " but no high γ -globulin. Mantoux was negative. A biopsy of the art. temporale failed to disclose arteritis. Urine culture was positive and streptomycin was tried but had to be stopped as the patient developed urticaria.

She was then in N until June 1973 (with the exception of dermatophytosis of the feet and cataract) when she was rehospitalized with fever, chills, pruritus and dysuria. Clinical examination revealed erythema, most obvious on the dorsal side of the feet, over the chest and on the face. There



Fig. 1 Electrophoretic migration pattern on paper

Fig. 2 "Atypical lymphocyte" (Sézary cell) in the peripheral blood of the patient. Giemsa, $\times 1000$

was no local edema, the local temperature was not increased and a fine furfuraceous desquamation could be seen over the same areas which were pruriginous. No palpable lymph nodes, liver or spleen were found. The laboratory findings were: Hb 11.9 g/100 ml, WBC 24000/mm³, differential count showed 3% eosinophils and 7.5% atypical lymphocytes, thrombocytes 510000/mm³, ESR 61 mm/h, serum iron 114 µg/ml, TIBC 309 µg/ml, B₁₂ 340 pg/ml, folic acid 2.3 ng/ml. The bone marrow showed normal erythropoiesis with normal sideroblasts but large iron deposits. The white granular series showed a moderate shift to the left and some more eosinophils. Some of the megakaryocytes were larger than normal. The conclusion of the laboratory was "reactive bone marrow" with reservation concerning the giant megakaryocytes as an argument in favour of a myeloproliferative syndrome. Rheumatoid factors were negative.

The immunoelectrophoresis showed on several occasions low values for all the immunoglobulins: IgG 0.3-0.4 g/100 ml, IgA 0.02-0.05 g/100 ml, IgM 0.03-0.05 g/100 ml, haaptoglobin 225-360 mg/100 ml.

Skin biopsy presented hyperkeratosis, parakeratosis, light acantosis. Dermal infiltration with a moderate number of rounded cells. The conclusion of the pathologist was: "Chronic eczema? No certain evidence of mycosis fungoides".

Reexamination of the peripheral blood smear by one of us then revealed that the atypical lymphocytes mentioned by the laboratory were in fact similar to the Sézary cells which we have seen in a previous case (11).

Blood samples showed the presence of 30% of lymphocytes with T cell characteristics and 8% with B cell characteristics. A normal stimulation by PHA and concanavalin A was found. Immunofluorescence techniques showed that 7.5% of the cells had immunoglobulins on their membrane surface. The patient's serum was also titrated against HSA-coated sheep RBC in passive agglutination giving a positive titer of 1:8000.

Several attempts were made to treat the urinary tract infection but on all occasions and with all antibiotics used she developed urticaria. An asthma-like accident was also noted while on nitrofurantoin. Attempted treatment of the Sézary syndrome was made as others did too with Leukeran® 11 mg/day and prednisolone 30 mg/day. The clinical impression was favourable, especially the effect on the pruritus, which disappeared, but the laboratory findings showed no changes and the treatment was stopped. She then began gradually to decline and developed signs of congestive heart failure although adequate amounts of digitals and diuretics were provided. A thrombosis in the left leg was followed by an abrupt fall in the thrombocytes and she finally died.

The post mortem diagnosis was: Congestive heart failure, endocarditis of aortic valve (old and new lesions), Several small emboli in the lungs, Thrombosis in the left vena iliac, Cystitis, Cholelithiasis. The lymph nodes were not enlarged, on the contrary rather hypoplastic. The spleen was described as "septic".

SOME CLINICO-PATHOLOGICAL ASPECTS OF THE SÉZARY SYNDROME

In the earlier reports there was a certain confusion about the clinical aspects of the disease, and one may feel that they included, under the same heading, true cases of Sézary malignant diseases from the lymphoma group and other dermatological diseases. As new cases were reported a clearer picture emerged and by now the clinical signs and symptoms which are consistent with the diagnosis can be divided into *Constant ones*: Erythroderma with exfoliation, Pruritus, Infiltration of the skin with "rounded cells", Presence of atypical lymphocytes in the blood, Normal bone marrow an important negative sign, *Intermediate ones*: Lymphadenopathy, Edema of the face ("leonine faces"), Hyperpigmentation (appears only by vitroprecipitation), Alopecia, Dystrophic nails (12), Hyperkeratosis of palms and soles, Hepatomegaly (12).

Biopsy of the skin

In nearly all the previously described cases there was great variation in the pathologist's diagnosis. Most frequently the skin was labelled as "chronic dermatitis" or "not

diagnosis: infiltration suggesting lymphoma" "mycosis fungoides on one arm, reticulosarcoma on the other" etc. The conclusion is that there is no specific lesion of the skin in the Sézary syndrome.

The Sézary cell

The atypical circulating cell is described as "mononuclear cell, with a large convoluted nucleus containing dense clumped chromatin and a scanty rim of cytoplasm. Mitotic nuclei or mitoses have been reported. Lutzner et al. (14) have described two types of cells, large and small. In another study Lutzner and Jordan (16) have shown by electron microscopy that the nucleus appears cerebriform,

with overlapping folds and clefts, containing serpentine chromatin, often interconnected by narrow bridges. The cytoplasm contains mitochondria, ribosomes, polysomes and small amounts of endoplasmic reticulum. Histochemically peroxidase, alkaline phosphatase and non-specific cholinesterase were noted as "unremarkable" (15) which is important for the differential diagnosis against normal or leukemic monocytes which present strong positive peroxidase and non-specific cholinesterase reactions. PAS-positive reactions in the cytoplasmic granules have been described but are not specific to the Sézary cell. Cytogenetic studies (15) have shown that the "large type" is hypo- or hypertriploid and the "small type" hyperdiploid or pseudodiploid. Another study (8) showed in one case three cell populations, one normal (10%), one containing 70-76 chromosomes and distinct marker (70%) and third with 86-100 chromosomes and different marker (20%).

All over the world there is now considerable interest in studies of T and B lymphocytes. Recently Broet et al. (4) using classical techniques but also an anti-T lymphocyte serum, were able to confirm in six cases that the Sézary cell bears T cell characteristics. U responsiveness of Sézary cells to PHA has also been reported (4).

Course and prognosis

The life expectancy in the Sézary syndrome is commonly five years from the time of diagnosis. There are very few reported post-mortem examinations (2, 3). Those available have not disclosed any specific modification in any organ, including lymph nodes or spleen. No metastatic lesions or leukemias have ever been found. Some imprints of lymph nodes are processed and revealed the presence of free Sézary cells. The alleged cause of death is usually congestive heart failure or "infections".

COMMENTS

The purpose of this paper is to describe the case of an 83-year-old woman with the Sézary syndrome. The diagnosis was based on the presence of erythrodermia, pruritus, presence of Sézary cells in the peripheral blood and in the skin together with a normal bone marrow.

We found that 30% of the patient's lymphocytes had T cell characteristics. Other recent studies (14) using an anti-T cell serum demonstrated that the

Sézary cells seem in fact to be T cells. They also co-operate with B cells in the production of various immunoglobulins. There is actually no proof that the Sézary cells behave abnormally but it may be so. Cottier et al. (7) recently proposed a new classification of lymph node pathology based on identification of T and B cells. This is a new approach and a study of Sézary would be interesting. The special features of our case worthy of comment were the hypogammaglobulinemia and the presence of antibodies against HSA.

Electrophoretic studies of serum from patients with Sézary syndrome have seldom been mentioned in the literature. Brody et al. (3) described a case as "unremarkable" and another as having "reduced serum albumin". Crossen et al. (8) described one case as "normal" while Broet et al. (4) found high IgM in one of six patients. We therefore consider the immunological deficiency of our patient remarkable. This was not present in 1964 but was fully developed in 1973. The choice had to be made between a primary deficiency of late onset (common type or "common variable") which is usually related to autoimmune disorders or a secondary deficiency. The latter is often found in patients suffering from lymphatic or other malignancies. In fact some authors (13) seem to consider the Sézary syndrome a rare chronic lymphatic leukemia. We think that this is rather speculative. As our patient failed to show any signs of leukemia or malignancy at post-mortem we consider that immunological deficiency was of primary type in this case. Normally the B lymphocytes are those which synthesize and secrete Ig. Some think that the T cells stimulate (directly) or co-operate with the B cells to this end. As Choy et al. (5) pointed out the decrease in Ig synthesis or failure of Ig secretion may be explained by a block in differentiation or maturation of B cells and subsequent arrest at a presecretory stage. We can but speculate that the Sézary cells have acted in this way.

The high titer against HSA is also remarkable and indicates break of tolerance. Animal experiments suggest that tolerance termination or circumvention can be caused by the administration of heterologous protein or homologous protein coupled to hapten. Antibody to thyroglobulin and subsequent thyrotoxicosis has been induced in rabbits by immunization with haptan-conjugated homologous thyroglobulin (18). In our case the drugs to which the patient reacted clinically may have functioned as a

happen, combining with the patient's albumin and resulting in a carrier conjugate. Alternatively tolerance termination can be induced by BSA in the food. Antibodies against BSA have been reported in rabbits after oral administration (10) but they are not a usual finding in man. Although BSA and HSA cross-react, human antibodies against BSA are normally directed only against antigenic determinants not present on HSA. (Unfortunately absorption studies could not be performed, as too little serum was left.)

Whether the Sézary cell itself is involved in this abnormal reaction against self-protein is left as an open question.

REFERENCES

1. Baccaredda, A., Arch. Derm. Syph. 179: 709 1939
2. Boyden V. J exp. Med 93 107 1951
3. Brody J, Cypress, E., Kimball, S. & McKenzie D. Arch Intern Med. 110: 705 1962
4. Brodet J., Flandrin G. & Seigman M. New Engl. J. Med. 289: 341 1973
5. Choy Y., Biggar W. & Good A. Lancet I 1149 1972.
6. Clendening W., Brecher O. & van Scott E. Arch. Derm. 89: 783 1964
7. Cottler H., Turk, L. & Sobin, L., WHO 47 375 1972.
8. Crosten P., Mellor J., Finley A., Ravich R., Vincent P. & Guai F. Amer J Med 40: 24 1971
9. Daniel, T., Flandrin G. & Le Jeune F. Nouv. Rev. franç. Hémat. 11 223 1971
10. Farr S. Fed. Proc. 19: 199 1970.
11. Hiescu, V. Unpublished data.
12. Jondal M., Holm G. & Wigzell H. J exp. Med 136: 707 1972.
13. Kadden R. Lancet I 688 1974
14. Labaze J., Moscovici A. & Pham, D. J. clin. Path. 25 312. 1972.
15. Lutzner M., Emerit H., Durepaire R., Flandrin G., Grupper Ch. & Premieras, M. J. natl. Cancer Inst 50: 1145 1973.
16. Lutzner H. & Jordan H. Blood 31 719 1968.
17. Sézary A. & Bouvrain Y. Bull. Soc. franç. Derm. Syph. 45 254 1938
18. Weigle O. J exp. Med 121 289 1965
19. Yam, T. Amer J clin Path 55 283 1971

EDITORIAL

SPLENECTOMY IN CHRONIC MYELOCYTIC LEUKAEMIA

Ever since the turn of the century the question of the advisability of splenectomy in cases of chronic myelocytic leukemia (CML) has been discussed with waxing and waning interest. Times when such a procedure has been advocated on more or less well founded arguments have alternated with periods when splenectomy has been condemned as contraindicated and potentially dangerous.

Reports have been published on occasional cases successfully subjected to this operation (1, 2, 5, 8, 9) and larger reviews have also tried to elucidate the accumulated experience in the literature (1, 7, 12).

On the whole, such reviews have resulted in a rather pessimistic view, as a large percentage of the operated patients seem to have gained very little benefit from the operation. Admittedly, these relatively early reports suffer from the fact that the operation at that time was usually undertaken as a last resort when the patient after conventional medical treatment had failed, was on a downhill course with severe anaemia, sometimes also leukopenia and bleeding tendency due to advanced thrombocytopenia (1).

As the general opinion seems to have changed considerably during the last few years and the operation is now performed under somewhat different indications in an increasing number of CML cases and even as an almost initial procedure, it may be in place to give an account of the recent results emerging from the literature and from congress reports.

The situation at present is quite different from what it was only a few decades ago. More effective antileukaemic drugs have made it possible to achieve a rapid initial remission in most cases of CML, there is a much greater possibility of coping successfully with infections that were often fatal before, the blood center service is much more elaborate today, not only as regards conventional blood transfusions but also in the selective transfer

of concentrates of e.g. platelets. All these means have helped to reduce the immediate postoperative mortality to acceptable levels and have led to the concept of elective surgery in suitable cases and when strict indications for the operation are otherwise present.

Thus the reluctance to perform splenectomy in CML felt by experienced investigators earlier (7, 8, 12) has decreased considerably during the last ten years or so. Many people have appreciated the philosophy of Crosby put forward in discussions over splenectomy in haematology in general: "Splenectomy should be done when the patient does not need it. If one waits until the patient needs the operation he may not be able to tolerate it" (3).

The earlier indications included massive splenomegaly with splenic infarction and/or unbearable discomfort from the enlarged spleen and the development of hypersplenism with pronounced anaemia and low platelet counts. In the splenectomized CML cases who survived the operation a rapid return of the red cell count to normal and an increase of the thrombocytes were regularly seen. In addition the patient's susceptibility to cytostatic drugs was also demonstrated to increase after the operation. Lower dosage of such drugs was often sufficient to keep the patient in a good condition and with a leukocyte count within the normal range. In exceptional cases all cytostatic treatment could be withdrawn for a considerable time after the operation (4).

To these indications can be added others that are even more important. The enormously enlarged spleen harbours a very great proportion, perhaps trillions, of the pathologic cells in the body. The operation removes this major fraction of cells in one step and if the patient has been treated initially with an appropriate course of antileukaemic drugs resulting in reduction of the spleen and the best possi-

ble general condition, further management may be considerably facilitated and a substantial prolongation of a remission can be anticipated with or without small doses of cytostatic drugs (1-10).

Zubrod has also suggested early splenectomy as a means of removing initially as much of the pathologic cell population as possible; thereafter he advocates intensive antileukaemic drug treatment in the hope that eventually all pathologic cells will be eradicated.

Furthermore, based on supporting evidence (11), it has been claimed that in the spleen a great number of malignant clones of cells can be found during the transition of a case of CML into the final acute leukaemia phase, so that the early removal of a potentially dangerous cell population might postpone the acute blastic crisis. This has been explained as the metamorphosis of a conditioned neoplasm into an autonomous one (10). More over the spleen can constitute an important hiding-place for leukaemic cells protecting them from the action of cytostatic drugs.

In Sweden an early splenectomy has been advocated for several years (1) as a means of better management of cases of CML and with the goal of facilitating the further control of the disease. A limited number of splenectomized cases supports the advisability of such a procedure with reference to the better state of the patient and a probably prolonged survival.

Spiers and co-workers in Britain (10) have recently reported their opinion regarding elective splenectomy early in the course of CML, based on a controlled pilot study of 17 cases which has already run for several years. They have formulated a number of reasons for undertaking the operation early in the course of the disease. They feel that there is a good case for early splenectomy in CML as a prophylactic measure against almost inevitable problems which will arise later in the course and as a means of certainly increasing both the quality and the length of the patients' life. Galton and Spiers (6) had already stressed that splenectomy is an excellent way of drastically reducing the total mass of leukaemic cells which also makes it possible to expose the patients to a minimum quantity of antileukaemic drugs later on. These have been

shown in some cases to affect adversely the further evolution of the disease (6). If it eventually turns out that the spleen is in fact a place for the development of new anaplastic clones of cells capable of initiating the transition of CML into acute leukaemia, additional advantage will be gained by an early operation.

Likewise Baikie, who earlier worked with Spiers in London, has performed early splenectomy in all cases of CML since 1965 in which no special contraindications were present. Although this was not a controlled trial, he claims that his preliminary results suggest a significant prolongation of life after the operation and he also holds that this procedure not merely removes the major extramedullary site of cell proliferation and cell accumulation, but also has a favourable influence on the natural history of the disease and especially the occurrence of metamorphosis or even acute transformation.

Now that an extensive controlled multicenter trial has been started under the auspices of the British Medical Research Council with the aim of evaluating the effects in cases of CML of early splenectomy on the length and quality of the patient's survival, haematologists all over the world await the results with eager interest.

Ragnar Berlin, Linköping, Sweden

REFERENCES

- Berlin, R. *Acta med. scand. Suppl.* 252, 1951.
- Canabon, G. P., Nordland, J. & Carbone H. P. *Cancer* 29: 660, 1972.
- Crosby W. H. *New Engl. J. Med.* 286: 1252, 1972.
- Curling, H. O. *Arch. intern. Med.* 120: 356, 1967.
- Dubois-Ferrière, H. & Radier J. C. *Schweiz. med. Wochr.* 97: 182, 1967.
- Galton, D. A. G. & Spiers, A. S. D. *Progr. Haematol.* 7: 343, 1971.
- Holt, J. M. & Witts, L. J. *Quart. J. Med.* 35: 369, 1966.
- Meeker W. R., de Perio, J. M., Grace, J. T., Stutzman, L. & Mittelman, A. *Surg. Clin. N. Amer.* 47: 1163, 1967.
- Schwarzzenberg, L. et al. *Brit. med. J.* 1: 700, 1973.
- Spiers, A. S. D. *Brit. med. J.* 2: 578, 1973.
- Spiers, A. S. D. & Baikie, A. G. *Brit. J. Cancer* 22: 192, 1968.
- Strumia, M. M., Strumia, P. V. & Bassett, D. *Cancer Res.* 26: 519, 1966.

NEUTROPHIL GRANULOCYTE FUNCTION IN THE EARLY DIAGNOSIS OF ACUTE MYELOMONOCYTIC AND MYELOBLASTIC LEUKAEMIA

Class O Solberg, Aksel Schreiner, Kjell B. Hellum and Erik Hamre

From the School of Medicine, Medical Department B, University of Bergen, Bergen, Norway

Abstract The phagocytic and bactericidal activities of neutrophil granulocytes from 5 patients with early acute myelomonocytic or myeloblastic leukaemia and 5 controls have been examined. In each patient the bactericidal activity was lower than in any control and the neutrophil dysfunction was demonstrated before leukaemia could be diagnosed from clinical and haematological findings. During periods of remission, the bactericidal activity was normal. Results of neutrophil granulocyte function studies may be significant aid in the early diagnosis of acute myelomonocytic and myeloblastic leukaemia.

In many cases the diagnosis of acute myelomonocytic or myeloblastic leukaemia is easy. The clinical picture calls for haematological examination which reveals the typical findings of anaemia, thrombocytopenia and large numbers of blast cells in the peripheral blood and bone marrow. However, during the early stage of the disease, when clinical symptoms are less pronounced, haematological data may only show minor alterations from normal values and the diagnosis may remain uncertain for months. In these cases we have found the results of neutrophil granulocyte function studies to be significant diagnostic aid.

One of us has developed a method which facilitates a precise *in vitro* evaluation of the phagocytic and bactericidal activities of neutrophil granulocytes (18, 19). Using this method, a significant reduction in neutrophil granulocyte function has been demonstrated in each of 5 patients with early acute myelomonocytic or myeloblastic leukaemia when the diagnoses based on clinical and haematological findings were inconclusive.

MATERIAL AND METHODS

Patients and controls

Leucocytes were obtained from 3 male and 2 female patients, aged 18-54 years, during early acute myelomonocytic (3 patients) or myeloblastic leukaemia. The diagnoses

based on clinical and haematological findings could not be substantiated until 3-11 weeks (mean 6) later. Three patients had experienced fever and malaise for 1-3 weeks and two patients sore throat and lymphadenitis for 1-2 weeks. In addition to fever, tonsillitis and lymphadenitis, clinical examination revealed signs of anaemia in 2 patients. Haematological data, including results of 3 peripheral blood smear and buffy coat examinations and 3 ternal bone marrow aspirate examinations from each patient, did not substantiate the diagnoses (Table 1). Auer bodies were not observed. Results of alkaline phosphatase reaction (6, 9) in neutrophils and PAS staining (6) in blast cells were within normal limits. Serum uric acid estimation (17, 22) was performed in 4 patients and the results were normal. Normal peroxidase reaction (10) was demonstrated in 40-60% of peripheral neutrophil granulocytes, the remaining peripheral myeloid cell showing little or no reaction. Segmented neutrophils and neutrophil precursors in bone marrow smears also showed diminished peroxidase reaction.

Leucocytes obtained from staff members of the Department were used as controls for comparison of phagocytic and bactericidal activities, and for each test with patient leucocytes a control test was performed.

Leucocytes

Leucocytes obtained by Isopaque®/dextran sedimentation of heparinized venous blood (10 U heparin/ml blood) were washed twice in heparinized saline (1 U heparin/ml saline) by centrifugation at 300 g for 5 min (18). A differential count was performed after the final centrifugation and the cells were resuspended in Hanks balanced salt solution containing 0.1% gelatin to make concentrations of 10^7 neutrophils (band-form and segmented) per ml.

Bacteria

Staphylococcus aureus Oxford (Heathley strain, obtained from the National Collection of Type Cultures, Colindale, London 1958) was used as the test organism (18). The bacteria were cultured overnight in Penassay broth (Difco) washed twice in 0.45% saline and suspended in Hanks balanced salt solution to an optical density of 0.6 at 620 nm in a Beckman spectrophotometer. This suspension was diluted in Hanks balanced salt solution containing 0.1% gelatin to concentration of $13 \cdot 10^7$ (17 $\times 10^7$ colony forming units per ml).

Table 1 Laboratory data from 5 patients with early acute myelomonocytic or myeloblastic leukaemia (mean values within parentheses)

Peripheral blood	
Hb concentration (g/100 ml)	7.3-14 (11.3)
Erythrocyte count (mm^3/mm^3)	2.4-4.9 (3.9)
WBC/ mm^3	3 200-13 500 (7 900)
Differential count (%)	
Blast cells	0
Myelocytes	0-13 (*)
Granulocytes	42-69 (56)
Lymphocytes and monocytes	23-40 (35)
Thrombocyte count/ mm^3	128 000-564 000 (308 000)
Sternal bone marrow aspirates	
Normocellular*	6/10
Slightly hypercellular*	4/10
Blast cells (%)	1-8 (4)
Promyelocytes (%)	3-10 (6)
Myelocytes and metamyelocytes (%)	31-70 (52)
Myeloid-erythroid ratio	4-19 (8.4)
Megakaryocytes present	10/10

No of aspirates/total no of aspirates

Serum

Volumes of 1 ml pooled fresh normal serum from 7 adults were stored at -30°C immediately before each experiment. 1 ml freshly thawed serum was mixed with 3 ml Hanks' balanced salt solution containing 0.1% gelatin.

Leucocyte-bacteria suspension

Leucocyte suspension: 0.5 ml bacteria suspension 0.1 ml and diluted serum, 0.4 ml were added to 12×75 mm disposable plastic tubes. This provided about 3 bacteria per trophic granulocyte and a final concentration of 10%.

The tubes were incubated at 37°C with an end-to-end rotation to promote contact between bacteria and leucocytes. Samples were removed at prescribed intervals for determinations of the total number of viable bacteria and the number of viable intracellular bacteria. The bactericidal capacity of the granulocytes is proportional to the total number of bacteria killed and inversely proportional to the total number of viable bacteria or the number of viable intracellular bacteria (19). The number of bacteria phagocytized equals the number of viable intracellular bacteria plus the number of bacteria killed (19).

The total number of viable bacteria was determined after osmotic disruption of the leucocytes by adding 0.01 ml of the leucocyte-bacteria suspension to 1 ml distilled water. Quantitation of viable bacteria was made from appropriate dilutions of this suspension using a standard pour-plate technique.

The number of viable intracellular bacteria was determined by the technique of Solberg (18). 0.01 ml of the leucocyte-bacteria suspension and 1 ml Hanks' balanced salt solution containing 0.1% gelatin, 500 μg streptomycin, 500 U penicillin G and 2 mg phenylbutazone were incubated at 37°C for 15 min and centrifuged for 10 min at 500 g. The cellular pellet was washed twice in Hanks' balanced salt solution and resuspended in 1 ml distilled water for

osmotic disruption of the leucocytes. Viable bacteria were counted by the pour-plate technique. The results for each subject are recorded as the mean of 3 experiments, one on each of 3 consecutive days.

RESULTS

In the test with neutrophils from the controls a marked reduction in viable bacteria was observed, especially during the early phase of incubation and only 0.8-2.0% (mean 1.3%) of the bacteria remained viable after 120 min (Fig. 1). In contrast 4.8-11.9% (mean 7.3%) of bacteria in the tests with patients' neutrophils remained viable after incubation for 120 min and large numbers of viable bacteria were located intracellularly (Fig. 2) indicating normal phagocytosis but reduced intracellular killing of bacteria. When the patients' condition deteriorated and more immature cells appeared in the bone marrow and peripheral blood the difference in granulocyte function between patients and normal controls became even more pronounced and finally the number of morphologically normal granulocytes was reduced to such an extent that the test could not be carried out.

Following antileukaemic therapy 1 patient had two complete remissions and 2 patients one remission each. During periods of complete remission results of granulocyte function tests were normal. Two patients could be followed for several months by frequent granulocyte function studies and haematological examinations. Reduced granulocyte function was demonstrated as soon as immature cells appeared in the peripheral blood and, in one instance, several days before change in normal blood picture. Clinical symptoms were not present at the time of these early relapses.

DISCUSSION

Several syndromes characterized by chronic bacterial or fungal infections have been related to defects in the microbicidal capacity of neutrophil granulocytes (3, 5, 8, 12, 14, 15, 21). The first syndrome to be described was chronic granulomatous disease which is characterized by the occurrence in male children of dermatitis, lymphadenitis, hepatosplenomegaly, chronic suppurative infections and granuloma formation (4). Peru term defects in neutrophil bactericidal capacity with clinical mani-

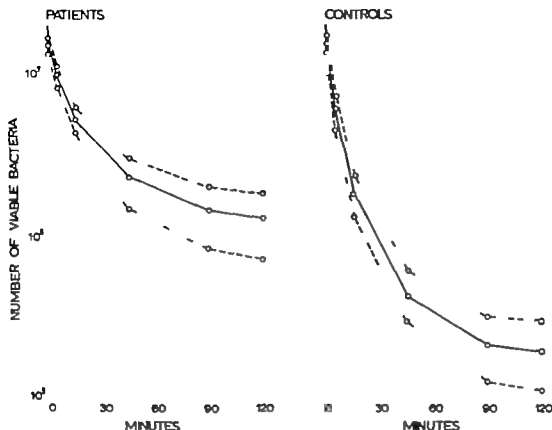


Fig. 1 Total number of viable bacteria during incubation with neutrophil granulocytes from 5 patients with early

acute myelomonocytic or myeloblastic leukaemia and from 5 controls. — = mean, --- = range.

festations of varying severity and with different genetic patterns have been included later in this syndrome (3, 8, 14, 21), indicating that the term chronic granulomatous disease covers several different types of neutrophil dysfunction. Myeloperoxidase (11, 12) and glucose-6-phosphate dehydrogenase (3, 14) deficiencies may also result in persistent neutrophil dysfunction and chronic infections. In addition, temporary dysfunction has been demonstrated, especially in patients with severe bacterial infections (13, 20) extensive burns (1, 2) and after acute trauma (1). Finally as demonstrated in the present study reduced granulocyte function may also be present during the early stage of acute myeloblastic and myelomonocytic leukaemia even before the diagnoses can be substantiated by clinical and haematological findings. Granulocyte function tests may therefore, be of significant value in the early diagnosis of these malignancies.

The differential diagnosis between disorders of persistent neutrophil dysfunction and early acute

leukaemia may sometimes be difficult. However the former disorders are usually inherited (3, 5, 8, 12, 14, 15, 21) and in chronic granulomatous disease the insidious onset during early childhood, the typical clinical picture of dermatitis, lymphadenitis, hepatosplenomegaly and chronic suppurative infections despite normal hematological findings should not be misinterpreted as acute leukaemia even in its very early stage. Besides neutrophil dysfunction is more severe in chronic granulomatous disease. In glucose-6-phosphate dehydrogenase deficiency hemolysis is a typical finding the myeloid series is normal and results of biochemical erythrocyte analyses will give the diagnosis (3, 14). In myeloperoxidase deficiency staining methods containing for example benzidine will demonstrate depletion of cytoplasmic myeloperoxidase (12, 14). Besides, these patients seem particularly susceptible to infection with *Candida albicans* (12, 14). Finally bacterial infection may sometimes be associated with transient neutrophil dysfunction (13, 20) and the

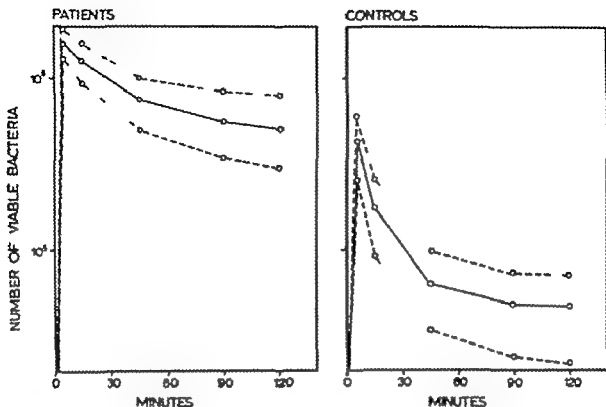


Fig. 2 Number of viable intracellular bacteria during incubation with neutrophil granulocytes from 5 patients with early acute myelomonocytic or myeloblastic leukaemia and from 5 controls. Symbols as in Fig. 1

blood picture may show leukaemoid reaction. In these cases results of neutrophil granulocyte function tests will soon improve and return to normal values during adequate antimicrobial therapy (20).

We do not know why neutrophil granulocyte function was reduced in our patients. The killing of phagocytized microorganisms by neutrophil granulocytes involves a complex series of biochemical events (5, 11, 12, 16) and it is likely that several interlocking killing mechanisms exist within each cell (5, 11, 16). However, as more information has been gathered during recent years correlating neutrophil bactericidal activity and neutrophil metabolic response to phagocytosis, it has become apparent that hydrogen peroxide plays an important role in the intracellular killing of microorganisms (5, 11, 14, 16). Between them myeloperoxidase, a halide and hydrogen peroxide form a strong antimicrobial system (11, 16) and in chronic granulomatous disease the bactericidal defect is characterized by reduced postphagocytic oxygen consumption and hexose-monophosphate-shunt activity resulting in reduced hydrogen peroxide

production (5, 11, 14, 16). The neutrophil dysfunction in glucose-6-phosphate dehydrogenase deficiency seems also to be due to deficient hydrogen peroxide production (3, 14) and in myeloperoxidase deficiency to absence of the enzyme itself (11, 12, 14). In diseases with temporary neutrophil dysfunction, intracellular enzyme defects have not been reported. However, in previous studies of patients with bacterial infections (20) we found low numbers of peroxidase-reacting granules more often in granulocytes from patients with reduced neutrophil granulocyte function than in patients with normal function. Leucocyte myeloperoxidase deficiency, *Candida* infection and reduced rate of intracellular killing of bacteria by the granulocytes have also been described in a patient with myelomonocytic leukaemia (7).

Whether the reduction in peroxidase-reacting granules demonstrated in the granulocytes from our leukaemia patients is the ultimate cause of the neutrophil dysfunction in early leukaemia or only part of a faulty differentiation of enzyme synthesis remains to be determined.

REFERENCES

1. Alexander J W, Windhorst, D. B. & Good, R. A.. Improved tests for the evaluation of neutrophil function in human disease. *J. Lab. clin. Med.* 72: 136, 1968.
2. Alexander J W & Wilson, D. Neutrophil dysfunction and sepsis in burn injury. *Surg. Gynec. Obstet* 130: 431 1970.
3. Bachner R. L., Johnston, R. B. Jr & Nathan, H. G. Comparative study of the metabolic and bactericidal characteristics of severely glucose-6-phosphate dehydrogenase deficient polymorphonuclear leukocytes and leukocytes from children with chronic granulomatous disease. *J. Reticuloendothel. Soc.* 12: 130 1972.
4. Bridges, R. A., Bernades, H. & Good R. A. A fatal granulomatous disease of childhood. The clinical study of new syndrome. *Amer J Dis. Child.* 97: 387 1979.
5. Cline, M. J. Leukocyte function in inflammation. The ingestion, killing and digestion of microorganisms. *Ser. Haemat.* 3: 3 1970.
6. Dacie, J. V & Lewis, S. M. Practical haematology pp. 74-91 Churchill, London 1966.
7. Davis, A. T., Brunning, R. D. & Quile, P. G. Polymorphonuclear leukocytes myeloperoxidase deficiency in a patient with myelomonocytic leukemia. *New Engl. J. Med.* 285: 789 1971.
8. Douglas S. D., Davis W. C. & Podenberg, H. H.. Granulocytopenias. Pleomorphism of neutrophil dysfunction. *Amer J Med.* 46: 901 1969.
9. Hayhoe, F. G. J. & Quaglini D. Cytochemical demonstration and measurement of leukocyte alkaline phosphatase activity in normal and pathological states by modified azo-dye coupling technique. *Brit. J. Haematol.* 4: 375 1958.
10. Kaplan L. S.. Simplified myeloperoxidase stain using benzidine dihydrochloride. *Blood* 26: 215 1965.
11. Klebanoff S. J. & Hanson, C. B. Role of myeloperoxidase-mediated antimicrobial systems in intact leukocytes. *J. Reticuloendothel. Soc.* 12: 170 1972.
12. Lehrer R. I. & Cline, M. J. Leukocyte myeloperoxidase deficiency and disseminated candidiasis. The role of myeloperoxidase in resistance to *Candida* infection. *J. clin. Invest.* 48: 1478, 1969.
13. McCall, C. E., Caves J., Cooper R. & DeChetdet, L.. Functional characteristics of human toxic neutrophils. *J. Infect. Dis.* 124: 68 1971.
14. Quile, P. G. Disorders of phagocyte function. Year Book Medical Publishers, Chicago 1972.
15. Quile, P. G., White J. O., Holmes, R. & Good R. A. *in vitro* bactericidal capacity of human polymorphonuclear leukocytes: Diminished activity in chronic granulomatous disease of childhood. *J. clin. Invest.* 46: 668, 1967.
16. Sharna, A. J., Padi, B. B., Jacobs, A. A., Strauss, R. R. & Mitchell, G. W. Jr. Role of host-parasite interactions. XXXVIII. Metabolic activities of the phagocyte as related to antimicrobial action. *J. Reticuloendothel. Soc.* 12: 109 1972.
17. Smoloffs, A. N. & Hartsell, S. E. The determination of serum lysozyme. *J. Bact.* 58: 731 1949.
18. Solberg, C. O. Enhanced susceptibility to infection. A new method for the evaluation of neutrophil granulocyte functions. *Acta path. microbiol. scand. Sect. B.* 80: 10, 1972.
19. — Protection of phagocytized bacteria against antibiotics. A new method for the evaluation of neutrophil granulocyte functions. *Acta med. scand.* 191: 383 1972.
20. Solberg, C. O. & Hellum, K. B. Neutrophil granulocyte function in bacterial infections. *Lancet* 2: 727 1972.
21. Thompson E. N., Cops, W. A., Chandra, R. K. & Southall, J. P. Leucocyte abnormality in both parents of patient with chronic granulomatous disease. *Lancet* 2: 799 1969.
22. Wiernik, P. H. & Serpick, A. A.. Clinical significance of serum and urinary myeloperoxidase activity in leukaemia and other haematologic malignancies. *Amer J Med.* 46: 330, 1969.

Medical Journals

Printed and distributed by Almqvist & Wiksell
publishers to the Universities of Uppsala, Stockholm and Göteborg
and to the Royal Swedish Academy of Science, etc

Acta Chirurgica Scandinavica

Editor: L. Thörn

8 issues per volume. Free supplements. Including free subscriptions to the *Scandinavian Journal of Plastic and Reconstructive Surgery* (without suppl.), the *Scandinavian Journal of Thoracic and Cardiovascular Surgery* (without suppl.) and the *Scandinavian Journal of Urology and Nephrology* (without suppl.).
Current volume 141/1975.

Sw kr 250 per volume, incl. postage

Acta Dermato-Venerologica

Editor: Nils Thyroson

6 issues per volume. Free supplements.
Current volume 55/1975

Sw kr 130 per volume, incl. postage

Acta Medica Scandinavica

Editor: J. Waldenström

6 issues per volume. Free supplements.
Current volumes 197-199/1975

Sw kr 225 per annum (two volumes), incl. postage

Acta Obstetrica et Gynecologica Scandinavica

Editor: Axel Ingelman-Sundberg

5 issues per volume. Free supplements.
Current volume 34/1975

Sw kr 150 per volume, incl. postage

Acta Oto Laryngologica

Editor: C. A. Hamberg

6 issues per volume. Free supplements.
Current volumes 79-80/1975

Sw kr 100 per volume. Two volumes per annum
kr 200 incl. postage

Pædiatrica Scandinavica

Editor: R. Zetterström

6 issues per volume. Free supplements.
Current volume 64/1975

Sw kr 175 per volume, incl. postage

International Journal of Fertility

Editor: S. J. Belzman

4 issues per volume.
Current volume 20/1975

Sw kr 120 per volume, incl. postage

International Journal of Gynecology and Obstetrics

Editor: Harold A. Kamrinsky

6 issues per volume.
Current volume 13/1975

Sw kr 110 per volume, incl. postage

Scandinavian Audiology

Editor: Bjørn Blegvad

4 issues per volume. Free supplements.
Current volume 4/1975.

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Infectious Diseases

Editors: Justus Ström and Jan Winblad

4 issues per volume. Free supplements.

Current volume 7/1975

Sw kr 110 per volume, incl. postage

Scandinavian Journal of Plastic and Reconstructive Surgery

Editor: Bengt Johansson

3 issues per volume. Free supplements.
Current volume 9/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Psychology

Editor: Lars Kebabian

4 issues per volume.

Current volume 16/1975

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Rehabilitation Medicine

Editor: Olof Håk

4 issues per volume. Free supplements.
Current volume 7/1975

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Rheumatology

Editor: Veljo Laane

4 issues per volume. Free supplements.
Current volume 4/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Social Medicine

Editor: Gunnar Loge

3 issues per volume. Free supplements.
Current volume 5/1975.

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Thoracic and Cardiovascular Surgery

Editor: Viking Olav Björk

3 issues per volume. Free supplements.
Current volume 9/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Urology and Nephrology

Editor: Åke Frick

3 issues per volume. Free supplements.
Current volume 9/1975.

Sw kr 100 per volume, incl. postage

Uppsala Journal of Medical Sciences

Editor: Gunnar Ågren

3 issues per volume. Current volume 80/1975

Sw kr 70 per volume, incl. postage

Free inspection copies on request—write to

The Almqvist & Wiksell Periodical Company
Box 62, S-101 20 Stockholm, Sweden

INTRAUTERINE DEATH AND CIRCULATING ANTICOAGULANT (ANTITHROMBOPLASTIN)

Inga Marie Nilsson Birger Åstedt, Ulla Hedner and
David Berezin

*From the Coagulation Laboratory and the Department of Obstetrics and Gynecology
University of Lund Malmö General Hospital Malmö, Sweden*

Abstract. A report is presented of a young, otherwise apparently healthy woman who had had three pregnancies which for some unknown reason terminated in intrauterine death (miscarriages or stillbirths). During the third pregnancy a coagulation defect was diagnosed which was characterized by prolonged coagulation times and prolonged one-stage prothrombin time. This defect disappeared after the end of the pregnancy but returned during the fourth pregnancy. This time circulating anticoagulant was found, which inhibited the action of thromboplastin. The values found for the various coagulation factors were normal. The anticoagulant titre rose during the pregnancy from 1/2 to 1/10. Leucocyte agglutinating as well as lymphocytotoxic antibodies directed against the husband's cells were demonstrated in the patient during the pregnancy. In this case by passage of cell fragments and thromboplastic substances to the mother, the foetus had probably induced the development of antibodies against the foetal tissues. The foetus may be regarded as an incompatible transplant. The fourth pregnancy was terminated by caesarean section in the 34th week. The child weighed 1440 g and, after three exchanges of blood, did very well. The placenta was severely infarcted. It is postulated that the development of antithromboplastin during pregnancy may be a contributory cause of intrauterine death.

Circulating anticoagulants may develop spontaneously even in the absence of primary haemophilia. Such anticoagulants have been demonstrated in patients with various autoimmune diseases such as SLE, rheumatoid arthritis, drug allergy in women after childbirth and sometimes in apparently healthy middle-aged and elderly persons (2, 4, 6, 9, 18, 21, 26). The anticoagulants may be directed against factors V, VIII, IX, XI, XII, prothrombin and thromboplastin. The occurrence of circulating anticoagulants is characterized by prolonged coagulation times and, clinically often by a tendency to haemorrhagic diathesis.

A description is given of a young woman who had had three pregnancies, each of which terminated in intrauterine death for which no explanation could be found. During the third pregnancy examination revealed a coagulation defect characterized by prolonged coagulation times and prolonged one-stage prothrombin time. This defect disappeared after the pregnancy but recurred during a fourth pregnancy. It was then found that she had a circulating anticoagulant inhibiting the action of thromboplastin. Thanks to careful observation and timely induction of delivery by caesarean section the woman gave birth to a healthy child.

METHODS

Platelet and coagulation studies. Determinations of the platelet count, Duke and Ivy bleeding times, platelet adhesiveness according to Hellén, whole blood method, coagulation time in glass and plastic tubes, recalcification time, partial thromboplastin time, one-stage prothrombin time (human brain thromboplastin), Stypven time, factor VIII, factor IX, factors XI and XII, prothrombin, factor VII and factor X (Owren's Pk-P test), factor V, fibrinogen, thrombin time and antithrombin III (biological and haemicochemical method) were made by the procedures described earlier (3, 12, 19, 24, 26).

Tests for circulating anticoagulant and normalization tests. The inhibiting effect of the patient's plasma on the recalcification time of normal plasma was determined. The highest plasma dilution capable of prolonging the recalcification time by 15 sec was taken as the anticoagulant titre of the patient's plasma. Normalization tests were carried out in the same way with the patient's plasma as test base (23).

In-o-stage methods for assay of inhibitors against factor VIII and factor IX. These tests were performed in the way described by Holmberg and Nilsson (14) and Nafsson et al. (20). **Inhibitory activity against factors XI and XII** was determined according to Åberg and Nilsson (26). **Anticoagulant activity in the presence of thromboplastin**

Table 1 Results of coagulation studies

	Third pregnancy week 31 11/68	Non-pregnant 7/69	Fourth pregnancy			
			Week 22 12/71	Week 27 1/72	Week 34 3/72	3 d. after delivery
Coagulation time (min)						
Glass	25	13	22	32	28	27
Plastic	60	34	>60	>60	>60	60
Platelet number (per mm ³)	120 000	290 000	176 000	240 000		
Platelet adhesiveness (%)						
Hellem	23	33	27			
Bleeding time (min)						
Duke		4	4			
Ivy			12	12		
Recalcification time (sec)	480	179	300	460	470	260
One-stage prothrombin time (sec)	21	16	20	22	20	18
Stypven time (sec)			33			
Partial thromboplastin time (sec)		90	180	287	320	218
P&P (%)	90	105	108	112		
Factor V (%)	95	74	86	92		
Factor VIII (%)	139	105	263	170		
Factor IX (%)			137	130		
Factor XI (%)			90			
Factor XII (%)			100			
Thrombin time (sec)	20	20	17			
Fibrinogen (g/100 ml)	0.49	0.36	0.55	0.51		
Anticoagulant titre		0	1 2	1 10	1 10	1 5
Antithrombin III (%)						
Biological method		100	120			
Imunochemical method		100	100			

The inhibiting effect of the patient's plasma on the coagulation time of normal plasma in the presence of various concentrations of human brain thromboplastin (prepared described by Owren (25) and Astrup *et al.* (1)) was determined. Normalization tests were done in the same way with the patient's plasma as test base. The clotting system: 0.2 ml normal citrated plasma or patient's plasma + 0.2 ml normal citrated plasma dil. 1/5 1/10 1/20 or 0.2 ml patient's plasma dil. 1/5 1/10 1/20 + 0.2 ml thromboplastin in decreasing concentrations (dil. 1/1 1/10, 1/100 1/1 000, 1/10 000) were mixed and incubated for 3 min in a water bath at 37°C, after which 0.2 ml 30 mM CaCl₂ solution was added and the clotting time was measured. The same tests were performed with Russell viper venom (Stypven Burroughs Wellcome & Co. London) instead of thromboplastin.

The thromboplastin generation test according to Baggs *et al.* (3) was modified so that Thromboflux (General Diagnostics) was substituted for the platelet suspension. The intrinsic thromboplastins formed using normal adsorbed plasma (dil. 1/5) and normal serum (dil. 1/10) was tested simultaneously on normal platelet-poor citrated plasma, patient's platelet-poor citrated plasma and a mixture of 4 parts normal and 1 part patient platelet-poor citrated plasma, respectively.

Fibrinolytic studies The following determinations were made: fibrinolytic activity of plasma and resuspended erythrocyte precipitate on fibrin plates, plasminogen (immunochromatological method), inhibitors of plasminogen activa-

tion (arokinase inhibitors), α_2 -macroglobulin and fibrin degradation products (FDP). The methods have been described previously (11, 13, 22).

Histocompatibility testing Lymphocytotoxic antibodies were determined according to Khammeyer-Nielsen and Kjerby (16) and leucocyte agglutinating antibodies according to Khammeyer-Nielsen (15).

CASE REPORT

A 25-year-old woman without heredity of haemorrhagic disease. In 1967 she had plethographically verified thrombosis of the right leg during use of oral contraceptives of combined type.

In 1966 the patient was pregnant for the first time she was then 19 years old. In the 31st week of pregnancy intrauterine death occurred and the patient was delivered of a macerated child 37 cm long. No explanation could be offered for the intrauterine death. Routine histological examination of the placenta revealed nothing remarkable.

In 1967 the patient was again pregnant: she was then 20 years old. Also that pregnancy terminated in intrauterine foetal death in the 22nd week. The patient was delivered of a severely macerated foetus 26 cm long. No explanation could be offered for the intrauterine death. Routine histological examination of the placenta showed widespread necrosis and calcareous deposits.

In 1969 the patient was pregnant for the third time she

Non-pregnant (mo. after delivery)

1 4/72	5 8/72	9 12/72	23 2/74	Normal range
38	32	28	36	6-14
53	54	50	100	12-32
			240 000	150 000-300 000
				17-33
				1-5
				6-12
280	230	220	300	120-180
17	16	16	21	13-15
			50	19
		160	250	90-120
			90	80-120
			113	60-140
			185	60-140
			95	60-160
				60-160
				60-160
				18-20
			0.34	0.20-0.40
1.5	1.2	1.1	1.2	
				60-140

was then 22 years old. In the 29th-30th week the patient complained of chest pain and thromboembolism was suspected. The diagnosis was not verified, but the patient was treated for short period of pregnancy with heparin and dicoumarol. The pains were afterwards localized to the region of the liver and the serum bilirubin rose to 1.5 mg/100 ml. An increase in diastase was noted as well as increased SGOT (310 U) and SGPT (500 U). Cholecystectomy revealed nothing remarkable. Intrauterine death occurred immediately after these episodes; the patient was then in the 34th week. She was delivered of slightly macerated foetus 34 cm long. Routine histological examination of the placenta showed widespread necrosis and abundant fibrin deposits. During her third pregnancy the woman also had Rh immunization (anti-c), but with such a low titre that it was not considered to be of clinical significance. During that pregnancy the coagulation status of the patient had been investigated. A coagulation defect was found with prolonged coagulation times in both glass and plastic tubes and prolonged one-stage prothrombin time, while the values found for the various coagulation factors were normal.

At reexamination 3 months after delivery both the coagulation times and the one-stage prothrombin time were normal.

In 1971 the patient was pregnant for the fourth time; she was then 24 years of age. She had her last menstruation on July 19 1971. Pregnancy was at first apparently normal. At examination in the 21st-22nd week the coagulation times

were again prolonged and were found to be due to an anticoagulant.

The patient was examined for any underlying disease. Examination for rheumatoid factors was negative. Antinuclear factor (ANF) was demonstrated in titre of 1/512. No LE cells could be demonstrated with certainty. Immunoelectrophoretic analysis revealed IgG 1100; IgA 120 and IgM 90 mg/100 ml all within normal limits. It was found that the patient's serum contained leucocyte agglutinating as well as lymphocytotoxic antibodies, which were directed also against the husband's cells. Leucocyte agglutinating antibodies were also demonstrated in amniotic fluid obtained by amniocentesis. Rh immunization against α was also demonstrated. Anti-c titre rose from 1/32 to 1/512 (papain). The patient, who belonged to blood group O Rh+ proved to be genotype CDE/CDE. The husband belonged to blood group Rh- with genotype cde/cde.

In the further course of the pregnancy the patient showed no signs of toxicosis. She had no oedema or proteinuria and the BP was normal. The excretion of oestriol in the urine was, however always somewhat low suggesting placental insufficiency. Biparietal measurements by means of ultrasonics showed also retarded growth. No treatment was given during pregnancy.

In the light of the earlier obstetric history and the above mentioned laboratory findings it was decided to deliver the patient between the 34th and 35th week of pregnancy. Caesarean section was done on March 17. The child, a girl with a birth weight of 1440 g, had an Apgar score of 9 points. The placenta showed abundant infarcts. The histological examination revealed signs of bleeding, necrosis and fibrin deposits.

The child was found to belong to blood group O Rh+ and Coombs test was positive. Serum bilirubin was 7.1 mg/100 ml and was not allowed to exceed 10 mg/100 ml. The three blood exchanges performed were uncomplicated and the further development of the child was normal except for some feeding difficulties. The child left hospital on April 20, 1972, and her development has since been normal. The mother had no complaints during the puerperium.

RESULTS

Coagulation studies

The results of the coagulation studies are summarized in Table I. At first examination during the third pregnancy (in 31st week) the platelet count was somewhat low. Platelet adhesiveness was normal. She had markedly prolonged coagulation times in glass and plastic tubes and prolonged recalcification and one-stage prothrombin times. The values for P&P factor V factor VIII fibrinogen and thrombin time were normal. At that investigation no tests were made for circulating anticoagulants. The patient had no signs of fibrinolysis and the values for the various components of the fibrinolytic system were normal (Table II).

Table II Data on the fibrinolytic system

	Third pregnancy 11/68	Non-pregnant 7/69	Fourth pregnancy			Non-pregnant (mo. after delivery)				Normal range
			Week 22 12/71	Week 34 1/72	Week 34 3/72	1	5	9	23	
Spontaneous fibrinolytic activity (lysed area in mm ²)										
Plasma	0	0	0	0	0				0	0-30
Resusp. euglob. prec.	0	38	0	0	0				0	0-70
FDP (μ g/ml)	0	0	0	0	35	10	0	0	6	0-5
Plasminogen (%)	131	91	95							60-140
Inhibitors of plasminogen activation (%)	67	11	114							60-140
α_2 -macroglobulin (%)	134	112	93							80-120

At reinvestigation 8 months later when the patient was not pregnant, she had a normal coagulation time, recalcification time and one-stage prothrombin time. No anticoagulant activity could be demonstrated.

During her fourth pregnancy the coagulation status was continually followed from the 22nd week of pregnancy. In the 22nd week she again had a prolonged coagulation time, recalcification time, one-stage prothrombin time and also Stypven time. Factor VIII, IX, XI, XII, V, prothrombin (P&P), fibrinogen levels were normal for the stage of pregnancy. She had a normal antithrombin III level and normal thrombin time. The patient's plasma in dilution 1/2 prolonged the recalcification and one-stage prothrombin times of normal plasma. Addition of normal plasma failed to normalize the prolonged coagulation times. These findings indicated an anticoagulant interfering with thromboplastin and/or the prothrombin activation. The anticoagulant titre increased during the pregnancy to 1/10.

The components of the fibrinolytic system were normal. FDP were not present until just before delivery. Three days after delivery the anticoagulant titre was 1/5. Nine months later the anticoagulant was still present, but the titre had decreased to 1/1. At investigation 2 years after the delivery the anticoagulant titre had increased to 1/2.

The components of the fibrinolytic system were normal. FDP were not present until just before delivery.

Three days after delivery the anticoagulant titre was 1/5. Nine months later the anticoagulant was still present, but the titre had decreased to 1/1. At investigation 2 years after the delivery the anticoagulant titre had increased to 1/2.

Properties of the anticoagulant

Addition of the patient's plasma to normal plasma increased the recalcification time and the partial thromboplastin time of normal plasma. The highest

dilution at which the inhibitory effect of the patient's plasma at the end of the pregnancy could be demonstrated was 1/10 in the recalcification system. As mentioned, addition of normal plasma to the patient's plasma did not shorten the prolonged coagulation times. Two-stage assays for inhibitors against factors VIII, IX, XI and XII showed no signs of an anticoagulant active against these factors. The effect of adding various amounts of human brain thromboplastin to the patient's plasma was investigated (Table III). The patient had a prolonged one-stage prothrombin time which became more pronounced when the thromboplastin was diluted. In the presence of undiluted thromboplastin the patient's plasma (3/73) prolonged the clotting time of normal plasma by 1-2 sec in dilution 1/1 with thromboplastin in dilution 1/10 by 5-10 sec in dilution 1/2 with thromboplastin in dilution 1/100 by 43 sec in dilution 1/5 with thromboplastin in dilution 1/1000 by 40 sec in dilution 1/10. For comparison, thromboplastin titration was performed on plasma from one patient with severe haemophilia A (<1% factor VIII) and on plasma from one patient with severe haemophilia A complicated by anti-AHF. Although the thromboplastin times were prolonged when thromboplastin was diluted, the changes were relatively small compared with those observed in the patient. The Stypven time was prolonged. The patient's plasma in dilution 1/2 prolonged the Stypven time of normal plasma from 20 to 29 sec. It was also shown that intrinsic thromboplastin obtained from normal reagents lost its activity if the patient's citrated plasma was used as a test substrate. As pointed out, the factor V and P&P levels were nor-

mal. She had no sign of a defect in the latest stage of the coagulation process and had a normal anti-thrombin III level. Taken together these observations indicate that the anticoagulant in this patient inhibited the conversion of prothrombin to thrombin and was probably directed against the action of both extrinsic and intrinsic thromboplastin.

The anticoagulant was demonstrated not only in plasma, but also in serum. The anticoagulant was not removed from plasma by adsorption with BaSO₄ or dialysis across a semipermeable membrane for 24 hours. The activity was unaffected by heating at 56°C for 30 min. When normal plasma and patient's plasma were mixed the anticoagulant activity was demonstrable already after 3 min.

The anticoagulant was found to be neutralized by antihuman IgG antiserum when tested according to Felestein et al. (8).

DISCUSSION

The patient was a woman who had had three earlier pregnancies all of which had for some unknown reason terminated in intrauterine death. During the fourth pregnancy Rh immunization against c was observed. During the first and second pregnancies no such immunization could be demonstrated and during the third it was barely demonstrable. There is therefore no reason to suppose that Rh immunization played any role in the cause of intrauterine death in the first three pregnancies.

During the third pregnancy a coagulation defect was diagnosed which was characterized by prolonged coagulation times and prolonged one-stage prothrombin time. At examination between the third and fourth pregnancy the coagulation status was normal. During the fourth pregnancy the coagulation time as well as the one-stage prothrombin time was

prolonged. It was demonstrated that the patient had a circulating anticoagulant. It did not inhibit factors VIII, IX, XI and XII. In spite of the prolonged one-stage prothrombin time and the prolonged Stypven time the values found for the factors in the prothrombin complex and for factor V were normal. The thrombin time, fibrinogen level and anti-thrombin III level were normal. No signs of abnormalities in the fibrinolytic system could be demonstrated. Addition of thromboplastin in decreasing concentration in plasma prolonged the clotting times much more than those of normal plasma, haemophilia A plasma and haemophilia A plasma with an anticoagulant against factor VIII. According to Margolis et al. (18) this is the pattern seen in patients with anticoagulants directed against the action of thromboplastin. The patient's plasma also inhibited intrinsic thromboplastin obtained from normal reagents.

Taken together the observations concerning the mode of action of the inhibitor indicated that the inhibitor interfered with the activation of prothrombin to thrombin by thromboplastin and most probably acted as an antithromboplastin. According to Deutsch (6) pathological inhibitors of blood coagulation may belong to two different categories according to their mode of action. One group of anticoagulants reacts with one of the clotting factors stoichiometrically in a bimolecular reaction and inactivates the clotting factor concerned. The other group interferes instantaneously in some unknown way with one of the later reaction steps in the activation of prothrombin without inactivating an individual clotting factor. It is quite clear that the anticoagulant of our patient belonged to the latter group. Deutsch (6) points out that patients with inhibitors of the second type rarely have bleeding symptoms. Our patient showed no signs of increased bleeding tendency.

Table III Effect of varying amounts of human brain thromboplastin

Source of plasma	Undiluted	1/10	1/100	1/1 000	1/10 000	Recalcification time
Normal	15	28	53	76	95	170
Patient plasma	20	44	120	220	287	470
Haemophilia A	15	31	59	101	197	460
Haemophilia A anticoagulant						
Normal plasma + patient plasma	15	31	64	106	207	600
ctrl. 1/5	15	29	90	150		248

Acquired circulating anticoagulants are inhibitors which have been characterized as immunoglobulins (7, 8, 9). As mentioned above, they may appear in various immunological disorders. The ANF titre was positive, but no signs of any underlying immunological disease of the patient could be demonstrated.

Circulating anticoagulants against factor VIII have been shown to be capable of appearing during pregnancy and to persist for a long time, causing severe haemorrhagic diathesis (23). In our patient the coagulation time was normal between the third and fourth pregnancy and the anticoagulant titre fell after the fourth pregnancy (Table 1). During the fourth pregnancy she was found to have also leucocyte agglutinating and lymphocytotoxic antibodies which were directed also against the husband's cells. This suggests that the pregnancy and the foetus probably owing to passage of cell fragments and thromboplastic substances to the mother's blood had induced the development of antibodies against the foetal tissues and acted as antithromboplastin in the coagulation process.

No signs of intravascular coagulation could be demonstrated, which may be explained by the assumption that thromboplastic substances passing to the maternal blood stream were neutralized by antithromboplastin existing there. This anticoagulant, not as mentioned, caused any bleeding. But it is quite feasible that the passage of circulating anticoagulants to the foetal blood stream with reaction to the foetal thromboplastin may cause bleeding with intrauterine death as a consequence. In view of the immunological situation demonstrated the foetus may thus be regarded as an incompatible transplant. It is very probable that a varying degree of incompatibility was present during the various pregnancies. In the last pregnancy the incompatibility was obviously not more severe than that the child could survive. But there were signs of placental insufficiency in the form of low excretion of oestriol in the urine and bi-parietal measurements with ultrasonics showed retarded growth. Owing to the laboratory findings and the observations made in the three earlier pregnancies the child was delivered by caesarean section in the 34th–35th week. The placenta was also full of infarcts and showed signs of bleedings and necrosis.

It is possible that in a case like this immunosuppressive treatment should be considered.

The favourable effect of such treatment has been shown in cases with circulating anticoagulants against factor VIII (10, 17) and factor IX (26).

As far as we know this is the first time that intrauterine death has been shown to be a consequence of the formation of antibodies acting as antithromboplastin. It shows that in women who have had a pregnancy terminating in unexplainable intrauterine death coagulation analysis is indicated with a special search for circulating anticoagulants.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (875-19X-87 11A) and Riksbanken Jubileumsfond.

REFERENCES

1. Astrup, T., Møller, S. & Hansen, J. R. The value of Owen's method of estimating prothrombin. *Scand. J. Clin. Lab. Invest.* 3: 209, 1951.
2. Bid, III, E. Acquired inhibitors of coagulants. *Ann. Rev. Med.* 20: 63, 1969.
3. Baggs, R., Douglas, A. S. & Macfarlane, R. G. The formation of thromboplastin in human blood. *J. Physiol.* 119: 89, 1953.
4. Corrigan, J. J. Jr, Patterson, J. H. & May, N. E. Incompatibility of the blood in systemic lupus erythematosus. A case due to hypoprothrombemia and a circulating anticoagulant. *Amer. J. Dis. Child.* 119: 365, 1970.
5. Cronberg, S. Investigation in haemorrhagic disorders with prolonged bleeding time but normal number of platelets. With special reference to platelet adhesiveness. *Acta med. scand. Suppl.* 486, 1968.
6. Deutsch, E. Acquired inhibitors in coagulation. In: *Coagulation* (ed. G. Scherer & P. E. Strandberg), p. 133. Academic Press, New York and London, 1973.
7. Feinstein, D. I., Rapaport, S. I. & Chong, M. N. Y. Immunologic characterization of factor VIII inhibitors. *Blood* 34: 85, 1969.
8. Feinstein, D. I., Rapaport, S. I., McObee, W. G. & Patch, M. J. Factor V anticoagulants. Clinical biochemical and immunological observations. *J. Clin. Invest.* 49: 1578, 1970.
9. Green, D. Spontaneous inhibitors of factor VIII. *Brit. J. Haematol.* 15: 57, 1968.
10. — A simple method for the purification of factor VIII (antithromboplastic factor) employing snake venom. *J. Lab. Clin. Med.* 77: 153, 1971.
11. Hedner, U. & Nilsson, I. M. Clinical experience with determination of fibrinogen degradation products. *Acta med. scand.* 189: 471, 1971.
12. — Antithrombin III in clinical material. *Thrombos. Res.* 3: 631, 1973.
13. Hedner, U., Nilsson, I. M. & Jacobsen, C. D. Demonstration of low content of fibrinolytic inhibitors in

- individuals with high fibrinolytic capacity. *Scand. J. clin. Lab. Invest.* 25: 329 1970.
14. Holmberg, L. & Nilsson, I. M. AHF related protein in clinical praxis. *Scand. J. Haemat.* 12: 221 1974.
15. Klemmeyer-Nielsen, P. Methods used in platelet and leukocyte immunology. *Progr. clin. Path.* 1: 161 1969.
16. Klemmeyer-Nielsen, P. & Kjærby, E. Lymphocytotoxic microtechniques. In: *Histocompatibility testing*, pp. 341-343. Munksgaard, Copenhagen 1967.
17. Lusher J. M., Iyer, R. & Evans, R. K. Effective suppression of factor 8 antibody in patients with haemophilia A with cyclophosphamide. VIIth Congr of the World Federation of Haemophilia, p. 74. Tehran Iran May 1971.
18. Margolis, A., Jr Jackson D. P. & Ratsoff O. D. Circulating anticoagulants. A study of 40 cases and a review of the literature. *Medicine (Baltimore)* 40: 145 1961.
19. Nilsson, I. M., Blombäck, M. & Ramgren, O. Haemophilia in Sweden. I. Coagulation studies. *Acta med. scand.* 170: 665 1961.
20. Nilsson I. M., Hedner U. & Björkén, H. Suppression of factor IX antibody in haemophilia B by factor IX and cyclophosphamide. *Ann. intern. Med.* 78: 91 1973.
21. Nilsson, I. M., Hedner U., Ekberg, M. & Dennerberg, T. A circulating anticoagulant against factor V. *Acta med. scand.* 195: 73 1974.
22. Nilsson, I. M. & Robertson, H. Effect of venous occlusion on coagulation and fibrinolytic components in normal subjects. *Thrombos. Diathes. haemorrh.* 20: 397 1968.
23. Nilsson, I. M., Skarseth, B. & Oydel, K. Circulating anticoagulant after pregnancy and its response to ACTH. *Acta haemat.* 19: 40, 1958.
24. Nye, S. W., Graham, J. B. & Brinkhous, K. M. The partial thromboplastin time as a screening test for the detection of latent bleedings. *Amer. J. med. Sci.* 243: 55/179 1962.
25. Owren, P. A. The coagulation of blood, investigations on a new clotting factor. *Acta med. scand.*, Suppl. 194 1947.
26. Åberg, H. & Nilsson, I. M. Recurrent thrombosis in a young woman with circulating anticoagulant directed against factors XI and XII. *Acta med. scand.* 192: 419 1972.

Table 1 Clinical and laboratory data on the patients in groups A and B

Pat. no.		Age (yr)	Sex	Weight (kg)	Hb (g/l)	MCV (fl)	MCHC (g/l)	Reticulo-cytes (10 ⁹ /l)	Bone marrow picture (%)	Blood smear	Spleen Size	Myel. metapl	Diagnosis
Group A													
1	♀	15		56	78	122	325	125	70 Ep 8 Mp 10 Nmc	Anisocytosis hypo-chromia, basophilic stippling	N	Not in	Paroxysmal nocturnal hemoglobinuria
2	♂	35		66	130	82	370	165	Not lev	Spherocytosis	(+)	Not lev	Hereditary spherocytosis
3	♀	22		65	104	76	330	30	42 Ep 15 Mp 15 Nmc	Spherocytosis	Spleen enlarged		Hereditary spherocytosis and iron deficiency
4	♂	23		52	81	109	320	480	86 Ep 5 Mp 5 Nmc	Anisocytosis, polychromasia, macro-spherocytosis	+	Not in	Aplastic or hemolytic anemia
5	♀	38		53	96	123	330	600	58 Ep 19 Mp 10 Nmc	Anisocytosis, polychromasia, basophilic stippling, acid red cells	Spleen enlarged		Hemolytic anemia (PNH like syndr. with morning hemoglobinuria and benzoideremia)
6	♀	44		55	139	105	320	700	55 Ep 15 Mp 11 Nmc	Polychromasia, acid red cells	+	Not lev	Hemoglobinopathy (16)
7	♂	23		65	159	102	350	50	26 Ep 20 Mp 18 Nmc	Spherocytosis	+	Not in	Hereditary spherocytosis
8	♂	68		69	73	112	340	250	35 Ep 20 Mp Nmc	Marked anisocytosis and polycytosis	++	+	Chronic myelogenous leukemia with secondary hemolytic anemia
Group B													
9 (I)	♀	40		67	104	117	330	70	10 Ep 35 Mp 15 Nmc	Some basophilic stippling	N	Not lev	Erythroblastopenia, on androgens, 0.5 mg theobromine, 100 mg/day
9 (II)				68	97	114	300	63	10 Ep 30 Mp 7 Nmc	Normal			Erythroblastopenia on androgens, 0.5 mg theobromine, 100 mg/day
10 (I)	♂	27		68	57	116	320	40	14 Ep 4 Mp 66 Nmc	Normal	N	Not lev	Aplastic anemia no treatment
10 (II)				68	70	85	320	9	Not lev	Normal			On androgens, 0.5 mg theobromine, 100 mg/day

	11	9	76	55	94	90	320	5	9 Ep 40 Mp 14 Nmc	Normal	(+)	O haemo- siderosis	p. pub anemia so treatment
	12	6	76	63	89	94	160	21	14 Ep 19 Mp 28 Nmc	Some anisocytosis	N	Not inv	Pulmonary adenoma, sec hypodysplasia so substitution
	13	9	20	49	119	101	320	70	31 (6) Ep 70 Mp 70 Nmc	Some anisocytosis	N	Not inv	Fibrosis, constitutional aplastic anemia, in re- sponse on androgen synthetone 100 mg/day
	14	9	63	67	83	113	330	85	60 Ep 21 Mp 6 Nmc	Anisocytosis poikilocytosis polychromasia	N	Not inv	Temporary bone marrow failure with polycy- themia after total gas- troct. polycythemia reborn vera. M. leuka.
	15	6	65	71	84	94	370	15	Dry tap	Normal	N	Not in	Aplastic anemia, so treatment
Group C	16	9	68	64	85	97	110	91	76 (6) Ep 16 Mp 6 Nmc	Marked aniso- poikilocytosis, polychromasia	++	Not inv	Sideroblastic anemia
	17	6	68	58	93	95	310	32	90 (15) Ep 10 Mp 14 Nmc	Marked aniso- poikilocytosis polychromasia	N	Not inv	Sideroblastic anemia developing into acute myeloid leukemia
	18	9	78	45	77	108	330	31	80 Ep 15 Mp 0.5 Nmc	Marked aniso- poikilocytosis polychromasia	N	Not inv	Sideroblastic anemia
	19	6	68	60	81	111	330	17	70 Ep 4 Mp (blau) 13 Nmc	Marked aniso- poikilocytosis polychromasia	++	Not	Acute myeloid leukemia
	20	6	70	99	60	170	364	18	48 (13) Ep 15 Mp 6 Nmc	Moderate aniso- poikilocytosis	+	Erythrocyto- phagocytosis some erythrop. retard.	Chron. glomerulonephr + Liver cirrhosis Treated with Cyto- some phosphate and prednisolone
	1	6	56	86	67	93	300	81	54 (2) Ep 17 Mp 8 Nmc	Marked aniso- poikilocytosis, polychromasia		Not inv	Sideroblastic anemia gradually developing to acute myeloid leukemia
	22	6	13	62	112	108	140	4	76 (14) Ep 21 Mp 10 Nmc	Moderate aniso- cytosis	(+)	Not in	Pern anemia in partial remission
	23	9	54	86	102	102	290	14	90 (22) Ep 13 Mp 8 Nmc	Moderate aniso- cytosis	N	Not inv	Sideroblastic anemia probably developing into acute leukemia

(Continued on next page.)

Table I (cont.)

Pat. no.	Sex	Age (y)	Weight (kg)	Hb (g/l)	MCV (fl)	MCHC (g/l)	Reti- culo- cytes (10 ⁹ /l)	Bone marrow picture (%)	Blood smears	Spleen Size	Myel metapl.	Diagnosis
24	♂	69	66	72	110	310	15	35 (3) Ep 40 Mip (mostly blast forms) 13 Nmc	Eos normal	N	Not inv	Refractory anemia developing into acute myeloid leukemia
25	♀	54	54	105	84	310	45	70 Ep 28 Mip 18 Nmc	Moderate eosino- philic cytoasts	++	Not inv	Stromablastic anemia developing into acute myeloid leukemia
<i>Group D</i>												
26	♀	73	51	104	90	300	9	25 Ep 26 Mip 4 Nmc	Leukoerythrobl.	+	Slight	Myeloid metapl.
27	♀	77	68	94	100	364	48	9 Ep 9 Mip 24 Nmc	Leukoerythrobl.	++	Slight	Myeloid metapl.
28	♂	67	66	81	84	290	90	53 Ep 70 Mip 1 Nmc	Leukoerythrobl.	++	Marked	Myeloid metapl.
29	♂	63	66	57	91	320	30	14 Ep 13 Mip 6 Nmc	Leukoerythrobl.	+	Not inv	Myeloid metapl.
30	♂	72	74	87	89	320	30	43 Ep 70 Mip 8 Nmc	Leukoerythrobl.	+++	Slight	Myeloid metapl.
31	♂	70	71	105	89	320	77	65 Ep 11 Mip 5 Nmc	Leukoerythrobl.	++	Moderate	Myeloid metapl.
32	♂	66	80	139	84	370	13	42 (18) Ep 15 Mip 35 Nmc	Normal	+++	None	Splenic lymphoma devel- oping into chronic lymphatic leukemia

splenomegaly six of whom had myelofibrosis (myeloid metaplasia) and one had malignant lymphoma restricted to the spleen. All patients were clinically stable and thought to be in steady state haematologically at the time of the study. Pertinent clinical and laboratory data are given in Table I.

METHODS

VCO was measured with closed rebreathing system according to the principles laid down by Coburn et al. (5, 6). After an equilibration period of 20 min blood samples were drawn every 15 min through an indwelling venous catheter. After 120 min (9 samples) 70.0 and 99.9% pure CO (AB Alfa, Malmo, Sweden) were injected into the system and final blood sample was drawn after another 25 min. The CO content of the blood samples was determined by gas chromatography as described by Cofhoun et al. (7-24) modified as described by Lundh et al. (19). 99.9% pure CO quantitated as described by Celis et al. (3), was used for calibration. The Hb concentration was measured according to van Kampen and Zijlstra (17). The absolute VCO (ml/min STPD) was calculated from the formula

$$\text{VCO} = \Delta\text{COHb} \cdot \text{dilution factor}$$

where ΔCOHb is the increase in blood COHb level per hour determined from the COHb of the first 9 blood samples, using the least square criterion to compute a best fit for the relation between time and COHb. The dilution factor was determined from the increase in COHb ($\Delta\text{COHb}_{\text{inj}}$) after the injection of known amount of CO (CO_{inj}) into the rebreathing system. The total CO binding capacity was calculated from the dilution factor

$$\text{CO binding capacity} = \frac{\text{CO}_{\text{inj}} \cdot 100}{\Delta\text{COHb}_{\text{inj}}}$$

Total body hemoglobin (TBHb g) was obtained by dividing the CO binding capacity by 1.39 and the total body haem (TBH mmol) by dividing TBHb by 16.1. The results of the VCO determinations are given in $\mu\text{mol/day}$ and in $\mu\text{mol}/\text{mmol TBH}$ and day. The S.D. for sample analysis of COHb calculated from duplicate determinations on 100 samples with COHb varying between 11.46 and 4.61% was found to correspond to a coefficient of variation of 3%. Duplicate analyses were always performed. The variation of VCO from month to month within the same individual was calculated from 20 determinations in seven healthy males with mean VCO of 1.3 $\mu\text{mol CO}/\text{mmol TBH}$ and day. The S.D. from month to month for each individual was found to be 2.85 μmol , corresponding to a coefficient of variation of 23%. The coefficient of variation for the regression coefficient used for the calculation of VCO was found to be 18% in the controls and 1% in the 37 patients.

Normal values. VCO measured in 20 young healthy males fasting in the morning after at least 36 hours free from CO exposure was found to be 0.55 ± 0.3 (mean \pm S.D.) ml/min, corresponding to 285 ± 340 $\mu\text{mol CO}/\text{day}$. Calculated in $\mu\text{mol CO}/\text{mmol TBH}$ and day the VCO in

this group was found to be 10.8 ± 5.5 (range 4.0-16.0). The initial baseline COHb in this group was $0.45 \pm 0.20\%$ (range 0.30-0.64) and the TBH 53.5 ± 14.8 mmol, corresponding to 1.20 ± 1.8 g Hb/kg b.wt.

Labelling of erythrocytes with ^{51}Cr was carried out according to method C from the ICSH Panel on Diagnostic Applications of Radioisotopes in Hematology (15). After correction for the elution of chromium according to the above mentioned recommendations, the data are plotted in linear diagram. The red cell survival was calculated from a straight line fitted to all the data points. If the fit was not satisfactory the survival was calculated from a straight line through the first few points only.

Vheme-c was obtained by dividing total circulating Hb in mmol (obtained from the dilution of the ^{51}Cr -labelled red cell) by the red cell survival time in days.

Routine hematology was performed with the aid of Coulter-Counter type S. Reticulocytes were counted according to Björkman (1) and serum bilirubin was measured according to Gombos and Schreiber (11). Bone marrow smears were usually obtained by sternal puncture and stained with May-Grienswald-Giemsa 1 on staining was performed according to Weinfeld and Hansen (28).

RESULTS

Group A

All patients in this group were known to have hemolytic anemia. The bone marrow picture was dominated by normoblasts and the reticulocyte count was elevated (Table I). All these patients had a high VCO (Table II) absolutely ($\mu\text{mol}/\text{day}$) as well as relatively ($\mu\text{mol}/\text{mmol TBH}$ and day). In six patients the VCO was compared to Vheme-c calculated from the red cell survival and large variations were found. In two patients patient 1 with a firm diagnosis of PNH and patient 5 who fulfilled some of the PNH criteria the VCO was much less than the Vheme-c suggesting heme losses or heme catabolism without corresponding CO formation. In patient 3 splenectomized due to hereditary spherocytosis and now iron deficient due to severe menorrhagia and with only a moderate decrease in red cell survival and in patient 6 whose hemolytic anemia was caused by a hemoglobinopathy the VCO/Vheme-c quotient was around 1.3. In patient 8 an elderly male with severe Coombs test negative hemolytic anemia added to the final stages of a chronic myelogenous leukemia, the VCO was found to be almost double the value derived from erythrocyte turnover (VCO/Vheme-c 1.87). Patient 7 with hereditary spherocytosis was found to have a similar increase in VCO compared to red cell survival (VCO/Vheme-c 1.76).

Table II Carbon monoxide production and hemoglobin catabolism

Pat. no.	TBHb %Cr (mmol heme)	TBH CO dilution (mmol)	Red cell Hr span %Cr (d.)	Vhemo-c (μmol/d.)	VCO		VCO/ Vhemo-c quotient	Serum bilirubin (μg/ml)	COHb (initial sample)
					μmol/d	μmol/mmol TBH and d.			
Group A									
1	14.1	16.3	17	835	510	31.6	0.61	10.0	1.05
2	25.6	30.4	30 ^a	850	1 350	45.7	1.68	8.0	0.90
3	27.8	30.0	60	470	608	20.2	1.30	13.0	0.99
4	17.3	21.7	9	1 700 ^a	1 600	74.0	0.94	49.0	1.70
5	26.2	21.5	9	2 220	1 650	77.0	0.74	25.0	1.70
6	34.0	37.7	17	2 040	2 795	83.2	1.37	60.0	1.70
7	30.8	53.6	70	725	1 280	23.8	1.76	15.0	1.27
8	27.8	27.9	39	715	1 320	44.8	1.82	13.0	1.50
Group B									
9 (I)	22.9	29.0	68	317	394	13.4	1.17	7.0	0.65
9 (II)	24.6	35.2	90	266	480	13.6	1.80	8.0	0.44
10 (I)	12.4	21.0	123	100	181	8.9	1.81	3.0	0.46
10 (II)	Not inv	21.6	Not inv	-	490	21.6	(4.90)	-	0.61
11	19.7	24.3	37	530	610	24.5	1.15	5.0	1.09
12	20.3	27.1	143	135	234	8.6	1.34	4.0	0.54
13	19.3	23.9	89	213	618	25.8	2.90	9.0	0.76
14	30.1	29.0	49	620	383	13.1	0.62	7.0	0.77
15	17.8	20.7	51	356	555	26.7	1.56	4.0	0.65
Group C									
16	18.1	28.6	70	230	745	28.6	2.98	9.0	0.70
17	22.4	29.2	64	345	820	27.8	2.38	8.0	0.77
18	12.4	18.5	73	174	468	25.2	2.69	8.0	0.88
19	24.9	26.1	83	374	975	38.0	2.60	4.0	1.15
20	21.4	29.9	22	970	2 430	82.0	2.51	19.0	1.57
21	20.4	25.3	33	612	1 570	60.2	2.56	13.0	1.25
22	39.0	46.5	136	290	770	17.2	2.66	15.0	0.66
	33.9	33.1	67	358	995	30.0	2.78	9.0	0.87
	21.0	24.6	100	210	586	24.2	2.80	4.0	0.81
	21.7	25.9	71	304	715	27.6	2.37	7.0	0.68
Group D									
26	19.4	22.9	88	213	352	15.4	1.65	5.0	0.47
27	22.6	36.4	39	590	1 240	34.6	2.10	8.0	0.62
					(1 050)		(1.78)		
28	30.8	43.0 ^a	30	1 050	2 180	50.0	2.04	8.0	1.70
					(1 850)		(1.76)		
29	15.2	14.4	44	330	533	37.2	1.62	11.0	0.72
					(615)		(1.75)		
30	56.0	59.3	68	840	1 130	19.0	1.35	7.0	0.72
31	29.0	30.8	53	1 265	1 635	43.8	1.31	8.0	1.90
32	68.1	79.8	97	704	892	11.2	1.27	8.0	0.64

Estimated from TBH (CO dilution) Fig. 4 Calculated from the reticulocyte count. ^a Probably erroneously high or low values. Figures within parentheses = corrected values.

Group B

The patients in this group were characterized by a hypoproliferative anemia and all except patient 14 had hypo- or acellular bone marrow pictures (Table I). The absolute VCO in these patients was usually low (Table II). In relation to TBH and day VCO was normal or moderately increased. In four patients the VCO/Vhemo-c quotient varied between

1.15 and 1.56 while in three the quotient was high (≥ 1.8) suggesting an increase in extraerythrocytic CO production. Two of the latter patients (no. 9 with erythroid blastopenia and no. 13 with Fanconi's constitutional aplastic anemia) were on pharmacological doses of androgens while the third (no. 10 study I) a young male with idiopathic aplastic anemia was not receiving any form of

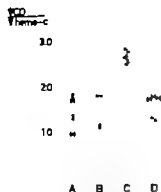


Fig 1 VCO/Vheme-c ratio in 8 patients with hemolysis (group A) 7 patients (one of whom was studied twice) with hypo- or aplastic bone marrow (group B) 10 patients with refractory anemia and hypercellular bone marrow (group C) and 7 patients with splenomegaly (6 of whom had myeloid metaplasia) (group D). Circles within parentheses in group A indicate ratios where Vheme-c was estimated from the reticulocyte count and in group B ratios corrected for probably erroneous TBH values \times = patients on pharmacological doses of androgens.

treatment. In patient 14 the VCO/Vheme-c quotient was very low (0.62) suggesting some heme loss (catabolism) without CO formation, an assumption corroborated by the finding of a continuous melæna secondary to thrombocytopenia.

Group C

This group consists of ten patients with anemia in combination with a hypercellular bone marrow dominated by erythropoietic sometimes megaloblastic cells and a low reticulocyte count (Table I). Absolute VCO was increased in all patients and the values were sometimes very high. One of the highest values of the study was seen in patient 20 a young boy with chronic glomerulonephritis and liver cirrhosis with splenomegaly treated with cyclophosphamide and prednisolone. In addition to the high cellularity his bone marrow picture was characterized by a marked erythrocytophagocytosis. Even though red cell survival was short, 22 days heme catabolism calculated from red cell survival represented only 40% of total heme catabolism (VCO/Vheme-c quotient 2.5) in this case. Since average red cell survival in this group was only moderately shortened, the high VCO resulted in VCO/Vheme-c quotients between 1.5 and 3.0 suggesting a marked increase

in CO production unrelated to circulating red cells in all cases in this group (Fig. 1).

Group D

This group consists of seven patients with splenomegaly six of whom had myelofibrosis (myeloid metaplasia) and one a malignant lymphoma restricted to the spleen. The diagnosis of myelofibrosis was supported by findings of a hypoproliferative anemia with splenomegaly and myeloid metaplasia in liver and spleen and a leukocrythroblastic peripheral blood picture. Absolute VCO varied from a low value (patient 26) to very high values (patients 28 and 31). There were however large variations in the red cell survival too from an essentially normal value (patient 25) to rather short values (patients 28 and 31). When CO production and red cell survival were compared in the VCO/Vheme-c quotient, two patients (nos 27 and 28) were found to have high values, 2.10 and 2.04 which should indicate an increase in erythrocytic heme catabolism. However when compared to body weight and Hb concentration, the TBH determinations in these two patients seem to have given erroneously high values. Since an erroneous TBH value will result in a falsely absolute VCO—whereas the relative VCO ($\mu\text{mol}/\text{mmol}$ TBH and day) will not be affected—the VCO/Vheme-c quotients in these two patients may be misleadingly high. In a third patient (no 29) the

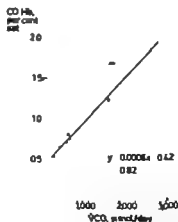


Fig 2 Comparison between the COHb in the arterial blood sample and the absolute VCO ($\mu\text{mol}/\text{day}$) in 32 patients with hematological disorders. Two patients were studied twice.

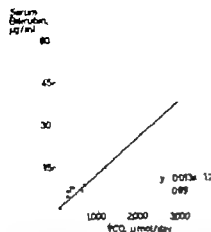


Fig. 3 Comparison between the concentration of unconjugated serum bilirubin and the VCO ($\mu\text{mol/day}$) in 31 patients with hematological disorders. One patient was studied twice.

TBH seems to be falsely low. In the patient with malignant lymphoma restricted to the spleen, which weighed 6 kg at splenectomy, the VCO/Vheme-c quotient was close to unity.

General

The relation between baseline COHb and absolute VCO ($\mu\text{mol/day}$) is seen in Fig. 2. The correlation is highly significant ($r=0.82$, $n=34$). There was also a significant correlation between the concentration of reacting bilirubin and absolute VCO ($r=0.69$, $n=34$) (Fig. 3). A comparison was also made between TBH measured with CO dilution technique and total circulating Hb mass measured after labelling red cells with ^{51}Cr (Fig. 4). The linear regression of this relationship follows the equation

$\text{TBH } \mu\text{mol heme} = (1.02 \text{ TBHb} + 4.35) \text{ } \mu\text{mol heme}$

with a correlation coefficient of 0.97. Three determinations with the CO technique were probably not correct (see Results, group D) and are not included in the calculation.

DISCUSSION

The original studies on VCO (5, 6) suggested only small inter- and intra-individual variations but further investigation has not corroborated these findings (21). Consecutive studies on some of the young men forming the present control group verified a large variation within the same individual from week to week. More than 50% of this varia-

tion is due to the rather large error in the measurement of VCO. However, when expressed as a percentage of the regression coefficient, the error is inversely proportional to VCO and is usually around 10% when the relative VCO ($\mu\text{mol}/\text{mmol TBH}$ and day) is increased by 100% of the normal or more, which was the case in 77 of the 32 patients in the present study.

Measuring red cell survival with the aid of labelling erythrocytes with ^{51}Cr is hampered by the fact that the isotope disappears from the blood not only through the demise of the erythrocytes but also through elution from the cell. Two main problems arise: firstly, there may be individual variations in the elution rate (4); secondly, this elution will affect the accuracy of the measurement mainly when red cell survival is normal or slightly decreased. When VCO is compared with data on the daily turnover of red cell hemoglobin (Vheme-c) calculated from red cell survival, allowance must be made for the fact that VCO represents the heme catabolism of the hours during which the study is performed, while the estimation of red cell survival is based upon observations made over several weeks.

Another reservation concerns the difficulty in obtaining uniform groups of patients. A common diagnosis does not guarantee that the patients will yield similar data in all variables. The only way

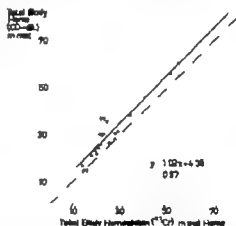


Fig. 4 Comparison between (anaemic) measurements of TBH estimated with the CO dilution method, and the circulating TBHb estimated with the ^{51}Cr dilution method in 30 patients with hematological disorders. Circles within parentheses are not included in the calculation of the regression line, since when compared to body weight and Hb concentration the TBH value was thought to be unreliable. One patient was studied twice.

— identity line, — regression line

round this problem is to present a lot of data on each patient, so that the reader may form an opinion by himself

Studying a group of normal males Coburn et al (6) found the mean VCO/Vheme-c ratio to be 1.27. Even though this figure is calculated from a small material it seems reasonable that total heme catabolism should exceed catabolism of red cell Hb by 30%. The true figure may be even higher, since Coburn's group used the TBH value obtained by CO dilution and not the TBHb obtained by ^{51}Cr dilution. Correcting for this difference, the VCO/Vheme-c ratio in Coburn's material becomes 1.44. Although we have no isotope red cell survival studies in our control group it can be shown indirectly that the figure given must be fairly correct. In our control group the mean TBH was 53.4 mmol. From the regression line in Fig. 4 it will be found that this corresponds to 48 mmol circulating Hb heme. A normal turnover of this heme will cause the formation of $48\,000/120 = 400\ \mu\text{mol CO/day}$. The mean daily absolute VCO in the group was 590 μmol corresponding to a VCO/Vheme-c ratio of 1.48. Accordingly a third of the daily heme turnover must derive from sources other than circulating red cells. In hemolytic anemia of not too complex nature as in hereditary spherocytosis the figure might be smaller because the portion corresponding to the turnover of red cells increases substantially. Even though it is known that the "early labelled" peak of bilirubin is increased after phlebotomy and in hemolysis (23) it has not been shown that the relation between effective and ineffective erythropoiesis is changed in the direction of a higher degree of ineffective erythropoiesis. Coburn et al. (6) calculated the VCO/Vheme-c ratio in seven patients with hemolytic anemia and found it to be 1.40, not significantly different from their reference value of 1.27 and close to our calculated reference value of 1.5.

The patients in group A are not a uniform group as can be seen from the diagnostic data in Table I. This may explain the variations in the VCO/Vheme-c ratio in this group. Two values were clearly below unity indicating heme losses without CO formation. One was from patient 1 with PNH. The intravascular hemolysis characterizing this disease is known to result in heme losses via the urine (14), which might result in a low VCO/Vheme-c ratio. The other low value was found in a female patient (no. 5) studied on many occasions during the

last 20 years in various hospitals without demonstration of any hemoglobinopathy enzyme deficiency or membrane abnormality. However she had morning hemoglobinuria and increased amounts of hemosiderin in her urine and she may represent a partial PNH syndrome even though many of the PNH tests (Ham's, Crosby's, and inulin tests) were negative. In patients 2 and 4 red cell survival studies were not performed. However daily turnover of red cells can be estimated at least semi-quantitatively from the reticulocyte count corrected according to Finch (10) assuming that the patients are in steady state. It will then be found that the daily Hb heme turnover represented 4 and 10% which gives VCO/Vheme-c ratios of 1.7 and 0.94 respectively. Consequently even though the range is wide the average ratio among the patients with hemolytic anemia is around 1.5, the calculated normal mean value.

The patients in group B had in common a hypoproliferative anemia with hypo- or acellular bone marrow pictures and pancytopenia. The majority had a VCO/Vheme-c ratio around the calculated normal value of 1.5 (1.15–1.80). The very low value comes from a patient with pancytopenia and continuous melanoma through the study period which explains her shortened red cell survival without any simultaneous increase in CO formation. The very high value 2.90 is from female (patient 13) with Fanconi's constitutional aplastic anemia, given oxymetholone 150 mg daily. Two other patients (no. 9 studies I and II and no. 10 study II) were also on pharmacological doses of this drug. Even though the second study of patient 10 was not accompanied by a red cell survival study it can be seen that the VCO/Vheme-c ratio in patients given oxymetholone is within the calculated normal range or higher. There are no signs of any substantial decrease in VCO during oxymetholone therapy as was suggested by Lundh et al. (20). In studies of the VCO/Vheme-c ratio in patients with sickle cell anemia given androgens in pharmacological doses. The difference might be due to the fact that the sickle cell anemia patients were given testosterone enanthate, but it is more probable that the assumption was based on too small a material. If testosterone has any effect on the VCO/Vheme-c ratio, it should be an increase since testosterone is known to induce the synthesis of short-lived heme-containing enzymes in the liver (1).

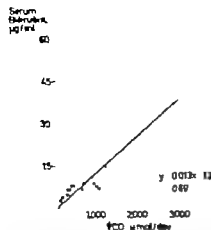


Fig. 3 Comparison between the concentration of unconjugated serum bilirubin and the VCO ($\mu\text{mol/day}$) in 31 patients with hematological disorders. One patient was studied twice.

TBH seems to be falsely low. In the patient with malignant lymphoma restricted to the spleen, which weighed 6 kg at splenectomy, the VCO/Vheme-c quotient was close to unity.

General

The relation between baseline COHb and absolute VCO ($\mu\text{mol/day}$) is seen in Fig. 1. The correlation is highly significant ($r=0.82$, $n=34$). There was also a significant correlation between the concentration of rectal reacting bilirubin and absolute VCO ($r=0.69$, $n=34$) (Fig. 3). A comparison was also made between TBH measured with CO dilution technique and total circulating Hb mass measured after labelling red cells with ^{51}Cr (Fig. 4). The linear regression of this relationship follows the equation

$\text{TBH (mmol heme)} = (1.02 \text{ TBHb} + 4.35) \text{ mmol heme}$

with a correlation coefficient of 0.97. Three determinations with the CO technique were probably not correct (see Results, group D) and are not included in the calculation.

DISCUSSION

The original studies on VCO (5, 6) suggested only small inter- and intraindividual variations but further investigation has not corroborated these findings (7). Consecutive studies on some of the young men forming the present control group verified a large variation within the same individual from week to week. More than 50% of this varia-

tion is due to the rather large error in the measurement of VCO. However, when expressed as a percentage of the regression coefficient, the error is inversely proportional to VCO and is usually around 10% when the relative VCO ($\mu\text{mol/mmol TBH}$ and day) is increased by 100% of the normal or more, which was the case in 27 of the 32 patients in the present study.

Measuring red cell survival with the aid of labelling erythrocytes with ^{51}Cr is hampered by the fact that the isotope disappears from the blood not only through the demise of the erythrocytes but also through elution from the cell. Two main problems arise: firstly, there may be individual variations in the elution rate (4); secondly, this elution will affect the accuracy of the measurement mainly when red cell survival is normal or slightly decreased. When VCO is compared with data on the daily turnover of red cell hemoglobin (Vheme-c) calculated from red cell survival, allowance must be made for the fact that VCO represents the heme catabolism of the hours during which the study is performed, while the estimation of red cell survival is based upon observations made over several weeks.

Another reservation concerns the difficulty in obtaining uniform groups of patients. A common diagnosis does not guarantee that the patients will yield similar data in all variables. The only way

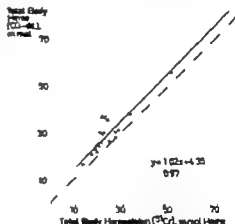


Fig. 4 Comparison between simultaneous measurements of TBH estimated with the CO dilution method, and the circulating TBHb estimated with the ^{51}Cr dilution method in 30 patients with hematological disorders. Circles within parentheses are not included in the calculation of the regression line, since when compared to body weight and Hb concentration the TBH value was thought to be unreliable. One patient was studied twice.
— = identity line — = regression line

ence is probably due to a higher degree of air pollution and CO contamination in urban areas of the USA. Accordingly if CO contamination can be excluded for more than 36 hours, the morning COHb will closely reflect total heme catabolism.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (Projects Nos. 19X-353 and 61P 3611), from the Medical Faculty University of Lund, from Selma and Augusta Svensson Fund for Cancer Research and from John and Augusta Persson Foundation for Scientific Research.

REFERENCES

- Björkman, S. E., Method for determining absolute reticulocyte count. *Scand. J. clin. Lab. Invest.* 10:435 1958.
- Cavallin-Ståhl, E., Berg, B. & Brandt, L. Reticulum cells and erythroblasts in the bone marrow of anemic patients. *Acta med. scand.* 195 183 1974.
- Colegin, M., Hansson, R. & Sundström, G. A sub-microtiter sampling device for quantitative collection of gases. Application to gas-liquid chromatographic analysis. *Scand. J. clin. Lab. Invest.* 27 367 1971.
- Cline, M. J. & Berlin, N. L. The red cell bromine elution rate in patients with some hematologic diseases. *Blood* 21 63 1963.
- Coburn, R. F., Blakemore, W. S. & Forster, R. E. Endogenous carbon monoxide production in man. *J. clin. Invest.* 42 1172, 1963.
- Coburn, R. F., Williams, W. J. & Kahn, S. B. Endogenous carbon monoxide production in patients with hemolytic anemia. *J. clin. Invest.* 45 460 1966.
- Collins, H. A., Roffey, F. L. & O'Neil, J. D. Determination of carbon monoxide in blood by gas chromatography. *Clin. Chem.* 14 162, 1968.
- Coburn, C. A. J. & Dudley, G. M. III. The relationship between endogenous carbon monoxide production and total heme mass in normal and abnormal subjects. *Amer. J. med. Sci.* 258 374 1969.
- Engstedt, L., Endogenous formation of carbon monoxide in hemolytic disease. *Acta med. scand., Suppl.* 332 1957.
- Fisch, C. Red cell manual. Hematology teaching manuals, p. 14 University of Washington, Seattle 1969.
- Gambino, S. R. & Schreiber, H. The measurement of bilirubin on the autoanalyzer by the method of Jendryaszek and Orf. *Technicon Symposium*, paper 34 1964.
- Granick, S. & Kappas, A. Steroid induction of porphyrin synthesis in liver cell culture. *J. biol. Chem.* 42:2587 1968.
- Hallberg, L. Blood volume, hemolysis and regeneration of blood in pernicious anemia. *Scand. J. clin. Lab. Invest., Suppl.* 16 1955.
- Hartman, R. C., Jenkins, E. J., McKee, L. C. & Heyssel, R. M. Paroxysmal nocturnal hemoglobinuria, clinical and laboratory studies relating to iron metabolism and therapy with androgens and iron. *Medicine* 45 331 1966.
- International Committee for Standardization in Hematology. Recommended methods for radioisotope red cell survival. *Blood* 38 378, 1971.
- Jeppsson, J. & Berg, B. Hemoglobin Lund 131 (H19) Oln-Oln in case of mild hemolytic anemia. In manuscript.
- van Kampen, E. J. & Zijlstra, W. G. Determination of hemoglobin and its derivatives. In: *Advances in clinical chemistry* vol. 8 (ed H. Sobotta & C. P. Stewart), p. 141 Academic Press, New York and London 1965.
- Lillemor, J. W. & Samra, M. I. The preleukemic syndrome. *Sem. Hematol.* 11 191 1974.
- Lundh, B., Johansson, M.-B., Merelid, C. & Cavallin-Ståhl, E. Enhancement of heme catabolism by caloric restriction in man. *Scand. J. clin. Lab. Invest.* 30:421 1972.
- Lundh, B., White, P. & Gardner, F. H. Effect of androgens on red cell mass and carbon monoxide production in sickle cell anemia (abstr.). *Clin. Res.* 17 334 1969.
- Lynch, S. R. & Moede, A. L. Variation in the rate of endogenous carbon monoxide production in normal human beings. *J. Lab. clin. Med.* 79 83 1972.
- Marver, H. S. & Schmid, R. The porphyrias. In: *The metabolic basis of inherited disease*, 3rd ed. (ed J. B. Stanbury, J. B. Wyngaarden & D. S. Fredrickson), p. 1087 McGraw-Hill New York 1972.
- Robinson, S. H. The origins of bilirubin. *New Engl. J. Med.* 279 143 1968.
- Roffey, F. L. & Collins, H. A. An artifact in the analysis of pyrenated blood for its low carbon monoxide content. *Clin. Chem.* 16 896 1970.
- Saarni, M. I. & Lillemor, J. W. Myelomonocytic leukemia. *Cancer* 77 72, 1971.
- Sjöstrand, T., Endogenous formation of carbon monoxide in man under normal and pathological conditions. *Scand. J. clin. Lab. Invest.* 1 201 1949.
- Wadman, B. Studies on erythropoietics in man. *Acta Univ. Upsal.* 142 11 1972.
- Weinfeld, A. & Hansen, H. A., Further studies on the interrelationships between hemolysis and sideroblasts in bone marrow smears. *Acta med. scand.* 171 23 1966.
- White, P., Coburn, R. F., Williams, W. J., Goldwirth, M. I., Rother, M. L. & Shaffer, B. C., Carbon monoxide production associated with ineffective erythropoiesis. *J. clin. Invest.* 46 1986, 1969.

NICOTINIC ACID AND THE ENDOGENOUS PRODUCTION OF CARBON MONOXIDE

B. Lundh, E. Cavallin-Ståhl and C. Merckle

From the Department of Medicine, University Hospital Lund, Sweden

Abstract. The endogenous production of carbon monoxide (VCO) has been followed with the aid of a rebreathing system for 3 hours in four healthy volunteers after injection of 30 mg nicotinic acid (NA). After an initial slight decrease for 15-30 min in the CO hemoglobin per cent saturation (COHb), rapid increase was registered for 170 min, whereafter the VCO returned to the normal preinjection level. The amount of "extra" CO produced varied between 4.1 and 11.1 ml corresponding to 9 and 1.6 g Hb or 182 and 98 μ mol heme, respectively. These figures are 3-5 times higher than those reported in the literature initiated from increases in serum iron, bilirubin and COHb (without the aid of rebreathing system). When related to the total body heme (TBH) estimated with the CO dilution technique the amount of "extra" heme metabolized after NA corresponded to 0.30% (range 0.26-0.32) of the TBH (a fourth of the total daily heme turnover or third of the daily Hb heme catabolism).

When Mattei (1) in 1946 reported that a nicotinic acid (NA) test gave rise to a temporary hyperbilirubinemia, he suggested that the effect was due to an increase in heme catabolism. This hypothesis was verified by Laurell (8) and Gydel (5, 6) who demonstrated a concomitant increase in serum iron and in carbon monoxide hemoglobin per cent saturation (COHb), respectively. The absolute amount of heme turned over in the NA test has not been known for certain. From calculations based on changes in serum bilirubin and serum iron and simultaneous determination of plasma volume, Gydel (5) calculated the amount of extra Hb broken down to 0.5 g, corresponding to twofold increase in normal Hb turnover for 72 hours.

A rebreathing system enables one to directly follow the amount of heme catabolized since all CO formed will be trapped and bound to the CO-binding pools in the body. Assuming that these pools are in equilibrium, measurement of changes in COHb

should give an accurate estimate of the speed and the absolute amount of extra heme turnover in the NA test.

MATERIAL AND METHODS

Twelve healthy males aged 31 and 40 years and two healthy females, aged 31 and 35 years, well informed about the objectives and means of the investigation volunteered for the study. They were known to be free from any hematological or hepatobiliary disease.

The endogenous production of carbon monoxide (VCO) was measured according to Coburn et al. (1). All studies were performed with the subject fasting between 8.00 and 1.00 a.m. Three of the subjects had not smoked for at least three years and the fourth, no. 4, smoked 10-15 cigarettes daily but abstained for 36 hours before the VCO study.

After 30 min equilibration period in the rebreathing system, blood samples were drawn every 1 min through an endwelling cannula catheter immediately after the fourth sample had been drawn. 40 mg NA was given over a period of 30 sec. Blood samples were then drawn for altogether 254 min (12 samples). Every 30 min another sample of 10 ml was drawn for determination of bilirubin immediately after the NA injection. The subject experienced burning sensation in the skin, especially in the face. This sensation subsided within few minutes. No other side-effects were noted.

The CO content of the blood was measured with gas chromatography as described by Rudkoy et al. (3, 14) modified as described by Lundh et al. (10). The Hb concentration was measured according to an Kampen and Zijlstra (7) and bilirubin according to Michaelson et al. (13). The CO binding capacity (COBC) was measured as described by Lundh et al. (10) in at least three separate determinations in each subject. Simultaneously the baseline production of CO (VCO_{baseline}) was measured. The mean value of these three determinations will be used in the calculations in the present study. Knowing the CO Hb C changes in COHb over a certain period (Δ COHb) can be used to calculate the VCO for this period using the formula:

$$VCO \text{ ml} = \Delta COHb \cdot COBC \cdot \Delta t$$

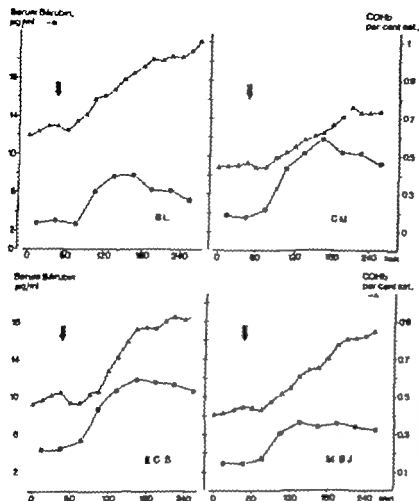


Fig. 1 Changes in COHb and serum bilirubin in four healthy volunteers after the NA test (arrow). The volunteers were kept in a rebreathing system throughout the study.

Furthermore, CO B C can be converted into total body Hb (TBHb) after dividing by 1.39 which in turn can be converted into mmol total body heme (TBH) after dividing by 16.1. The VCO related to NA (VCO_{NA}) was calculated from the total VCO during the period following NA injection (VCO_{total}) after subtracting the mean baseline VCO measured in at least three separate studies ($VCO_{baseline}$).

RESULTS

As will be seen from Fig. 1 the NA test resulted in marked changes in COHb in all subjects studied. Immediately after the injection there was a slight decrease varying between 0.01 and 0.06 COHb. The lowest point occurred at 15 or 30 min after the injection. There was then a rapid increase in COHb varying between 0.30 and 0.40 COHb. This rapid increase usually started before the 45th min after the injection and persisted for another 120 min after which COHb seemed to increase at the normal

(baseline) speed indicating a return to a normal production of CO.

Serum bilirubin was measured twice before the injection of NA without showing any significant changes. After the injection an increase was measured after 30 min in three of the cases and the maximum level was reached after 120 min, when an almost threefold increase was demonstrated in all cases. No significant amounts of conjugated bilirubin were found in any of the samples taken. The absolute amount of extra heme catabolized after the NA test was calculated from the measurements of VCO (Table I). The "extra" CO produced after the NA test varied between 4.10 and 1.80 ml representing 2.91 and 1.29 g Hb which corresponds to 182 and 80 µmol heme respectively. The latter value, from subject 2, is probably falsely low and it will be seen from Fig. 1 that the COHb was constant in four samples before the injection and in the last

Table 1 Carbon monoxide binding capacity (COBC) total body heme (TBH) total endogenous production of CO (VCO_{basal}) baseline endogenous production of CO (VCO_{baseline}) and the endogenous production of CO related to nicotinic acid (VCO_{NA})

Case no.	Sex	Age (y)	B wt. (kg)	COBC (ml)	TBH (mmol)	VCO_{basal} (ml)	VCO_{baseline} (ml)	VCO_{NA} (ml)	Extra heme catabolized		Extra Hb catabolized (g)
									μmol	% of TBH	
1	♂	40	82	1270	56.8	6.03	1.93	4.10	182	0.32	91
2	♂	31	65	1000	44.6	3.00	(1.20)	(1.80)	(80)	(0.18)	(1.29)
							0.00	3.00	134	0.30	2.13
3	♀	35	80	835	37.5	3.42	1.20	2.22	98	0.26	1.61
4	♀	31	54	730	32.7	3.43	1.04	2.39	106	0.32	1.71

Figures within parentheses, see Results

four samples. This indicates that the real VCO_{baseline} in subject 2 during the day of the NA test was not significantly different from zero. Correcting for this VCO_{NA} in subject 2 becomes 3.0 ml corresponding to ~ 13 g Hb or 134 μmol heme. When the extra heme catabolized is calculated as a percentage of the total body heme the values lie within the range of 0.26–0.32%.

DISCUSSION

The main advantage with a rebreathing system in heme catabolism studies is that it allows one of the metabolites to be collected completely without seriously affecting the equilibrium of the system. The error is rather large, around 20% for values of VCO within the normal range, but it is much smaller, 10% or less (9) when VCO is increased by 100% or more, which seems to be the case in the NA test.

All subjects in the present study had a normal VCO measured in three separate studies. The NA test caused an increase in serum bilirubin of the same degree as reported by Mattes (12), Gydel (5, 6) and Fromke and Miller (4) indicating that the data obtained from the CO studies may be used as a basis for calculating the amount of extra heme turned over in the NA test in normal man.

Even though the VCO_{baseline} was also calculated from the increase in COHb in the first four samples drawn before the injection, we find it more correct to use the mean VCO obtained from three independent VCO studies performed under basal conditions, especially as the study is then based on nine samples drawn during 120 min. However, this method was not used in our case 2 because his VCO on the day of the NA test did not differ significantly

from zero, a finding which serves to illustrate the large intrasubject day-to-day variation in VCO (9, 11). The results for case 2 are therefore given with the VCO_{baseline} assumed to be nil.

The results for the extra heme turned over in the NA test suggest an extra Hb turnover varying between 1.7 and 3.3 g. These are much higher values than those calculated by Gydel (5) from the increases in serum iron and serum bilirubin in the NA test and compared to the injection of non-viable red cells. However, the fact that Gydel could not exclude changes in the clearance of bilirubin and iron from plasma and did not follow his cases for more than 60 min might explain his much lower values. On the other hand it is quite clear from our study as well as from that of Gydel (5) that the amount of heme broken down in the NA test is related to the total Hb mass. The extra heme catabolized varied within a rather narrow range, 0.26–0.32% of TBH, mean 0.30%. These figures should be compared with the normal daily heme catabolism, which measured in our laboratory with the CO production technique amounts to 1.1% of the TBH pool in healthy young men. The extra heme metabolized in the NA test accordingly represents more than a fourth of the normal daily total heme turnover or a third of the daily turnover of circulating Hb heme. The use of a constant absolute dose of NA led to variations when the dose was calculated per kg body weight. However, no relation was found between the dose (mg/kg) and the amount of extra heme catabolized.

The decrease in COHb in the first two samples after the injection of NA might represent a changed equilibrium between the heme pools in the body elicited by the vasodilator effect of the drug. This effect might abate successively, leading to a

ration of the initial equilibrium something which has not been corrected for. If corrected for the values for extra heme catabolized should be 5–10% smaller.

The present study has not thrown any light on the mechanism behind the increased heme turnover induced by nicotinic acid. But we have shown that it is possible to directly measure the absolute amount of extra heme metabolized in the NA test and that this amount seems to be up to five times larger than suggested by earlier calculations from increases in serum iron and bilirubin.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (Projects Nos. 194-3523 and 61P-3611) and from the Medical Faculty University of Lund.

REFERENCES

1. Coburn, R. F., Blakemore, W. S. & Forster, R. E. Endogenous carbon monoxide production in man. *J. clin. Invest.* 42, 1172, 1963.
2. Coburn, R. F., Williams, W. J. & Kahn, S. B. Endogenous carbon monoxide production in patients with hemolytic anemia. *J. clin. Invest.* 45, 460, 1966.
3. Collison, H. A., Rodkey, F. L. & O'Neal, J. D. Determination of carbon monoxide in blood by gas chromatography. *Clin. Chem.* 14, 162, 1968.
4. Fromke, V. L. & Miller, D. Constitutional hepatic dysfunction (CHD: Gilbert disease): review with special reference to a characteristic increase and prolongation of the hyperbilirubinemic response to nicotinic acid. *Medicine* 51, 451, 1972.
5. Gydel, K. On the hyperbilirubinemic and hypersideremic action of nicotinic acid on normal subjects and on patients with some hematological disorders. *Acta med. scand.* 162, 9, 1958.
6. — Transient effect of nicotinic acid on bilirubin metabolism and formation of carbon monoxide. *Acta med. scand.* 167, 431, 1960.
7. van Kampen, E. J. & Zijlstra, W. G. Determination of hemoglobin and its derivatives. In: *Advances in clinical chemistry*, vol. 8 (ed. H. Sobotta & C. P. Stewart), p. 141. Academic Press, New York and London, 1965.
8. Laurell, C.-B. Influence of nicotinic acid on the intermediary metabolism of iron and bilirubin. *Acta pharmacol. toxicol.* 9, 86, 1953.
9. Lundh, B., Cavallin-Ståhl, E. & Mercke, C. Heme catabolism, carbon monoxide production and red cell survival in anemia. *Acta med. scand.* 197, 161, 1975.
10. Lundh, B., Johansson, M.-B., Mercke, C. & Cavallin-Ståhl, E. Enhancement of heme catabolism by caloric restriction in man. *Scand. J. clin. Lab. Invest.* 30, 421, 1972.
11. Lyoch, S. R. & Moede, A. L. Variation in the rate of endogenous carbon monoxide production in normal human beings. *J. Lab. clin. Med.* 79, 85, 1972.
12. Matti, C. Sui vari aspetti della curva bilirubinica da carico di acido nicotinico nei normali e negli epatopazienti. *Minerva med.* 37, 308, 1946.
13. Michellson, M., Norsten, B. & Sjölin, S. Plasma bilirubin determination in the newborn infant. A methodological study with special reference to the influence of hemolysis. *Pediatrics* 35, 925, 1965.
14. Rodkey, F. L. & Collison, H. A. An artifact in the analysis of oxygenated blood for its low carbon monoxide content. *Clin. Chem.* 16, 896, 1970.

OXYGEN UPTAKE AND CARDIAC OUTPUT DURING SUBMAXIMAL AND MAXIMAL EXERCISE IN ADULT SUBJECTS WITH TOTALLY CORRECTED TETRALOGY OF FALLOT

Björn Björke

From the Department of Pediatrics Karolinska Institute
St Göran's Children Hospital Stockholm, S edon

Abstract Ten female and eight male adults with tetralogy of Fallot, the majority totally corrected at adult age, have been studied at rest and during submaximal and maximal exercise on a bicycle ergometer. Oxygen uptake was determined by the Douglas bag technique and cardiac output by the dye-dilution method. Maximal oxygen uptake was reduced about 30-40% from normal. Thus a complete normalization of the aerobic working capacity was not achieved in spite of an intracardiac repair that was considered surgically satisfactory. Cardiac output response to exercise was subnormal, mainly due to small stroke volumes and partly because of low heart rates. A fall in stroke volume of more than 10 ml was found in 8 of the patients during exercise. No correlation was found between stroke volume during maximal exercise, on the one hand, and the presence of particular residual defect, anatomy of the right ventricular outflow tract prior to operation and the use of right ventricular outflow patch on the other. However, too few patients were studied to allow any definite conclusions as to the possible influence of these variables. It remains to be shown whether the haemodynamic abnormalities will be less and the aerobic work capacity better if total correction is undertaken at an early age.

Influenced by the age at which total correction is performed.

Several authors (9, 17, 18, 33, 40) have found normal or near normal haemodynamics at rest and during mild exercise in patients with technically good operation results. However, this does not mean that they have a normal exercise tolerance and a normal haemodynamic response to heavy work. In the haemodynamic studies cited above, most patients were corrected before the age of 10. The aim of this study was therefore to study the haemodynamic response during submaximal and maximal exercise in a group of patients with TOF corrected at adult or near adult age. The haemodynamic study presented in this article is part of a larger investigation comprising pulmonary and renal function as well as muscle metabolism in patients with TOF (5, 7, 8).

MATERIAL

Eighteen adults, 10 females and 8 males, with totally corrected TOF took part in this study. All belonged to the material previously studied by Möder (33) and all had undergone palliative shunt operation between 1947 and 1955 at a mean age of 5 years. Total correction was undertaken at 19.5 years of age (range 11-27.5). Only 3 patients were less than 15 years old when intracardiac repair was performed. The mean age at the follow-up was 26 (range 19-35). Since total correction 6.5 years (range 2.5-10.5) had thus elapsed. The anatomy classification suggested by Hare et al. (19) was used for the right ventricular (RV) outflow tract before operation. Eight patients had isolated subpulmonary stenosis (type I), 7 combined valvular and subpulmonary stenosis (type II) and 3 diffuse hypoplasia of the outflow portion of the RV and the main pulmonary artery (type III). Intracardiac repair was carried out in the Departments of Thoracic Surgery Karolinska Sjukhuset, Stockholm (14 cases) and in the University Hospital,

The first patient with tetralogy of Fallot (TOF) was totally corrected in 1955 (32). Thus the knowledge of the long-term results is incomplete. Total corrective operation has dramatically changed and improved the natural history for patients with TOF. Still, even a successful operation must be looked upon as an anatomical correction with some residual since the patients are left with a ventriculotomy scar, often with some degree of right ventricular outflow tract stenosis and a right bundle branch block (RBBB).

The question is whether these residuals will interfere with the normalization of the circulation which is aimed at when total correction is undertaken and whether the impact of these residuals is

Table I Individual values for oxygen uptake ($\dot{V}O_2$), cardiac output (\dot{Q}), heart rate (HR), stroke volume (SV) and blood lactate concentration at rest (R) in supine position and during submaximal (E_1 , E_2) and maximal exercise (E_{max}) in the sitting position

Pat. no.	Age (y)	Sex	Height (cm)	Weight (kg)	Blood vol (l)	Exercise state	$\dot{V}O_2$ (l/min)	\dot{Q} (l/min)	HR (beats/ml)	SV (ml)	Lactate (mmol/l)	Remarks*
1	28.5	♀	170	73.5	4.7	R	0.25	5.6	85	66	0.6	Type I VSD (minimal)†
						E_1	0.97	10.1	118	85	2.0	
						E_2	1.57	13.0	152	83	4.2	
						E_{max}	1.95	15.5	174	89	11.1	
2	25	♀	164	55	4.1	R	0.21	4.4	74	60	0.5	Type I —
						E_1	0.88	8.2	128	64	—	
						E_2	1.02	9.1	152	60	4.8	
						E_{max}	1.18	9.4	170	55	7.0	
3	22	♀	167	60.3	4.4	R	0.21	4.5	63	72	0.7	Type II PI
						E_1	0.93	9.1	117	78	3.2	
						E_2	1.36	10.1	156	65	3.6	
						E_{max}	1.61	13.4	173	78	6.0	
4	22	♀	167	48.4	4.7	R	0.11	3.9	66	59	1.5	Type II PI
						E_1	0.82	6.3	114	51	3.8	
						E_2	1.16	6.7	168	40	6.9	
						E_{max}	—	8.9	182	49	10.1	
5	27.5	♀	163	62.7	4.5	R	0.21	3.9	61	63	0.8	Type II PI
						E_1	0.84	7.3	99	73	5.3	
						E_2	1.33	8.5	145	58	5.0	
						E_{max}	1.48	9.1	178	51	10.8	
6	27	♀	163	48.4	4.2	R	0.25	4.3	71	61	0.6	Type I
						E_1	0.82	6.3	101	63	3	
						E_2	1.42	10.0	165	61	12.1	
						E_{max}	1.57	11.4	176	65	15.7	
7	23.5	♀	161	50.5	3.5	R	0.24	4.1	78	53	1.1	Type II PI
						E_1	0.61	6.5	121	53	3.0	
						E_2	0.85	7.3	149	49	4.8	
						E_{max}	1.08	7.9	171	46	9.7	
	35	♀	164	58		R	0.29	5.4	86	63	0.7	Type III PI (pronounced) + PS
						E_1	0.96	10.7	121	88	3.2	
						E_2	1.26	11.0	143	77	5.9	
						E_{max}	1.53	10.9	164	66	9.7	
9	24	♀	158	62	3.8	R	0.21	4.6	76	61	0.9	Type I VSD+PS+PP‡
						E_1	—	7.3	109	67	5.0	
						E_2	1.16	9.0	139	65	7.3	
						E_{max}	1.53	9.7	174	56	15.6	
10	1	♀	165	63	3.8	R	0.19	4.4	83	53	0.8	Type II PP‡
						E_1	0.79	7.2	109	66	2.0	
						E_2	1.27	9.5	143	66	3.1	
						E_{max}	1.68	11.7	186	63	7.9	
11	23	♂	189	67.5	6.3	R	—	6.3	79	80	1.0	Type III PS+PI+VSD (minimal)†
						E_1	1.49	10.5	121	87	3.3	
						E_2	1.97	12.5	150	83	5.8	
						E_{max}	2.34	13.4	177	76	12.8	
12	19	♂	168	67	5.0	R	0.26	5.3	66	81	1.1	Type I PS+VSD‡
						E_1	0.91	9.2	104	89	—	
						E_2	1.86	12.3	155	79	3.7	
						E_{max}	2.30	14.1	187	76	10.0	
13	25	♂	171	56.1	4.2	R	0.26	5.1	78	66	0.5	Type III‡ PS+PI
						E_1	1.05	8.7	129	67	3.1	
						E_2	1.44	11.4	168	68	6.0	
						E_{max}	1.66	12.4	185	67	14.0	

Table I (cont.)

Pat. no	Age (y)	Sex	Height (cm)	Weight (kg)	Blood vol. (l)	Ever close state	$\dot{V}O_2$ (l/min)	\dot{Q} (l/min)	HR (beats/min)	SV (ml)	Lactate (mmol/l)	Remarks*
14	29	♂	180	79	3.5	R	0.37	4.7	55	86	0.6	Type I
						E ₁	1.48	10.1	123	82	-	
						E ₂	1.90	17.5	156	80	3.9	
						E _{max}	2.11	14.1	179	79	7.4	
15	25	♂	160	61.2	3.8	R	0.3	5.7	57	99	0.8	Type I VSD (minimal)?
						E	0.88	9.0	84	108	2.1	
						E ₂	1.43	11.3	129	95	4.1	
						E _{max}	2.01	13.5	174	78	9.0	
16	31	♂	192	106	6.4	R	0.33	4.3	60	70	1.4	Type I
						E ₂	1.39	11.0	106	106	3.0	
						E ₂	2.04	14.8	145	102	6.5	
						E _{max}	3.3	13.7	161	85	10.4	
17	26.5	♂	175	60	4.9	R	0.29	6.4	75	85	0.9	Type II
						E ₂	1.44	9.9	120	83	-	
						E ₂	-	12.3	168	73	6.7	
						E _{max}	2.19	14.4	183	78	13.6	
18	30.5	♂	174	58.6	4.1	R	0.29	4.3	83	66	1.0	Type II PI
						E ₂	0.84	7.3	110	67	3.9	
						E ₂	1.39	9.6	172	46	9.8	
						E _{max}	1.70	10.4	184	48	14.4	

*Type of anatomy in right ventricle outflow tract prior to operation and residual defects.
Diagnosis established at cardiac catheterization.

Uppsala (4 cases) using patch in the ventricular septal defect (VSD) in all cases. An RV outflow tract patch was used in 10 patients and in one of them it was extended into the main pulmonary artery.

19 of the patients' postoperative haemodynamic assessment including cardiac catheterization had been performed elsewhere usually within one year after the operation (range 1 month-3 years) whereas the remaining patients unwilling to undergo another cardiac catheterization were investigated only clinically. Five patients were found to have no residual lesion according to the criteria stated under Methods. Another 2 patients had a minimal VSD with no detectable shunt. Thus 7 patients could be regarded as having excellent operative results. Three patients had VSD with small left-to-right shunt. Pulmonary valve stenosis (PS) was present in 5 patients and pulmonary valve incompetence (PI) in 10. The operative results were considered acceptable in all patients. All subjects were in sinus rhythm and none received any medication at the time of the study. Individual anthropometric data are listed in Table I as well as a detailed presentation of residual defects present.

PROCEDURE

The investigation was carried out in Goran Children's Hospital in March-Nov 1977. The patients were hospitalized for 3-4 days during the study. On the second day they performed a preliminary test designed to evaluate their aerobic working capacity. The actual exercise study

was performed in the morning of the third or fourth day after light meals. Polyethylene catheters 10 cm long, were introduced percutaneously into brachial artery and cubital vein. Simultaneous determinations of cardiac output, oxygen uptake, ventilation and blood lactate concentrations were done at rest in the supine position and during exercise in the sitting position. The exercise was performed on an electrically braked bicycle ergometer (Eliasm) at pedal rate of 60 rev/min. Two submaximal work loads corresponding to about 40 and 80% of maximal oxygen uptake were performed. Maximal exercise was also done at the highest possible work load the patient could perform for 3-7 min as judged by the information obtained from the preliminary test. The patients were allowed 5-10 min pause between the submaximal exercise and 15 min rest before maximal exercise. This was preceded by 3 min 'warming up' period corresponding to about 50% of maximal aerobic power. A blood volume determination preceded the exercise study. No adverse effects were noted in any patient during the study.

COMMENT

The 'levelling off' criterion (2) for maximal load on the oxygen transporting system was not strictly used in this study. However in the test preceding the actual study the intention was to establish the maximal load on which the subject worked to exhaustion in about 6 min. From this maximal load and subject

Table II Oxygen uptake ventilation cardiac output stroke volume heart rate haemoglobin and blood lactate concentrations at rest and during exercise in 10 female and 8 male corrected patients with TOF (means \pm S D)

Submax I submax II and maximal load averaged respectively 279 472 and 644 kpm/min for the female and 460 747 and 979 kpm/min for the male subjects

		Rest	Submax I	Submax II	Max. work
V_{O_2} (l/min STPD)	δ	0.28 \pm 0.03	1.18 \pm 0.29	1.72 \pm 0.28	2.08 \pm 0.27
	η	0.23 \pm 0.03	0.84 \pm 0.11	1.24 \pm 0.20	1.51 \pm 0.26
V (l/min BTPS)	δ	8.0 \pm 1.1	29.3 \pm 6.2	43.9 \pm 6.6	69.1 \pm 14.0
	η	7.6 \pm 1.9	25.0 \pm 3.6	36.9 \pm 6.0	56.2 \pm 12.0
R	δ	0.76 \pm 0.02	0.85 \pm 0.04	0.91 \pm 0.03	1.04 \pm 0.05
	η	0.81 \pm 0.08	0.85 \pm 0.02	0.91 \pm 0.04	1.01 \pm 0.05
\dot{Q} (l/min)	δ	5.3 \pm 0.8	9.4 \pm 1.4	12.2 \pm 1.4	13.2 \pm 1.3
	η	4.5 \pm 0.6	7.9 \pm 1.7	9.4 \pm 1.8	10.8 \pm 2.3
HR (beats/min)	δ	67 \pm 10	112 \pm 16	155 \pm 14	179 \pm 8
	η	74 \pm 9	113 \pm 9	151 \pm 10	175 \pm 6
SV (ml)	δ	79 \pm 12	86 \pm 18	80 \pm 15	74 \pm 9
	η	61 \pm 6	69 \pm 13	62 \pm 13	62 \pm 13
Hb (g/100 ml)	δ	13.8 \pm 1.2	14.5 \pm 1.6	15.4 \pm 1.5	15.9 \pm 1.8
	η	12.8 \pm 1.0	13.6 \pm 0.9	14.1 \pm 0.8	14.5 \pm 0.9
Lactate (mmol/l)	δ	0.9 \pm 0.3	3.1 \pm 0.7	5.8 \pm 2.0	11.4 \pm 2.6
	η	0.8 \pm 0.3	3.4 \pm 1.1	5.8 \pm 2.6	10.3 \pm 3.2

tive evaluation by the investigator as to whether this load really represented the individual's maximum, the load in the actual test was chosen. The lactate values obtained were >9 mmol/l in 14 of the 18 subjects investigated (1). It is therefore concluded at the mean deviation for maximal oxygen uptake in the 'true' value is marginal.

METHODS

Cardiac output (\dot{Q} l/min) was determined by the dye-dilution technique using indocyanine-green (Cardio-green®) as the indicator substance and a Beckman densitometer as the recording unit. The amount of dye which was injected with a calibrated syringe into the cubital vein varied from 0.80 to 1.73 ml depending on the size of the patients and the work performed, the concentration being 10 mg/ml. Arterial blood was drawn through a cannula inserted in the brachial artery at a constant rate of 55 ml/min. The blood was kept sterile and reinfused after each determination. Duplicate \dot{Q} determinations were made at rest and on each exercise level. Blood for 4-point calibration curves was obtained during maximal exercise in all subjects. The dye-dilution curves were calculated according to the method of Kinsmann et al. (29) including manual semi-logarithmic plotting, extrapolation and planimetry of the curve areas. The error of the method was calculated from the difference between two consecutive steady state determinations in which the mean difference in heart rate did not exceed 5 beats/min. Fifty-two pairs of observations were analysed varying from 3.9 to 15.5 l/min and the error was found to be 8.9%.

Heart rate (HR beats/min) was calculated from continuous ECG tracings, counting over a period of 15 sec. HR_{max} is defined as the value obtained at exercise breaking point.

Oxygen uptake (V_{O_2} l/min STPD) was determined using the Douglas bag technique and the volume of expired air (V l BTPS/min) measured in a calibrated Tissot spirometer. Fractions of O_2 and CO_2 in samples of expired air were analysed by the Micro-Scholander method (39).

Arterial haemoglobin concentration (Hb g/100 ml) was measured by the cyanmethaemoglobin method and haematocrit (Hct) with a high-speed centrifuge.

Arterial lactate concentration (lactate, mmol/l) was measured enzymatically (11). Total blood volume (l) was measured by the ^{51}Cr albumin method (43). Values obtained were corrected for the difference between body Hct and central Hct.

Heart volume (HV ml) was calculated according to Jones (25).

The presence of residual heart lesions was established from previous postoperative catheterization (9 patients) and/or from physical examination. Significant residual PS was considered present if a peak systolic gradient of ≥ 30 mmHg over the RV outflow tract and/or pressure in RV ≥ 50 mmHg was present at the follow-up catheterization. A PS was also said to be present if a $\geq 3/6$ ejection systolic murmur was detected over the pulmonary valve area. A VSD was considered to be present either if proven at catheterization or if pansystolic murmur (not due to tricuspid insufficiency) was found. PI was assessed by length and intensity of the diastolic regurgitant murmur.

The Wilcoxon rank sum test was used to test the possible influence of a particular residual defect and of the use of a

Table III Correlation matrix for maximal oxygen uptake and some dimensions and functional capacities of the oxygen transport system in 18 totally corrected adult patients with TOF

n=20 of observations

	$\dot{V}O_{2 \max}$ (l/min)	$\dot{V}O_{2 \max}$ (l/min/ kg b wt.)	\dot{Q}_{\max} (l/min)	SV _{max} (ml)	HR _{max} (beats/min)
BSA (m ²)	740 ^{***} n=17	-.058 n.s. n=17	.606 [*] n=18	.677 [*] n=18	-.289 n.s. n=18
V _{max} (l)	.512 [*] n=17	.567 [*] n=17	.403 n.s. n=18	.334 n.s. n=18	.169 n.s. n=18
HV (ml)	.486 n=17	-.019 n.s. n=17	.267 n.s. n=18	.322 n.s. n=18	-.234 n.s. n=18
Blood vol (l)	-.038 n.s. n=16	.489 n.s. n=16	.274 n.s. n=17	-.319 n.s. n=17	.260 n.s. n=17
\dot{Q}_{\max} (l/min)	.846 ^{***} n=17	.553 n=17	- n=18	.974 ^{***} n=18	.057 n.s. n=18
SV _{max} (ml)	0.816 ^{**} n=17	.422 n.s. n=17	0.974 ^{**} n=18	- n=18	-.167 n.s. n=18
HR _{max} (beats/min)	.153 n.s. n=17	.517 [*] n=17	.057 n.s. n=18	-.167 n.s. n=18	-
$\dot{Q}_{\max} \times 1.34 \times \text{Hb}$.841 ^{**} n=17	.903 n=17	.966 ^{***} n=18	.834 ^{**} n=18	0.87 n.s. n=18

right ventricular outflow patch on stroke volume and $\dot{V}O_{2 \max}$. Otherwise current statistical methods were used (41). The degree of probability was designated as follows: 0.05 < p not significant (n.s.); 0.05 > p > 0.01 probably significant (*); 0.01 > p > 0.001 significant (**); 0.001 > p highly significant (***).

RESULTS

Individual and mean values are given in Tables I and II and a correlation matrix in Table III.

The mean maximal oxygen uptake ($\dot{V}O_{2 \max}$) was 2.08 l/min for the males and 1.51 l/min for the females. This corresponds to a value of 30.5 ml/min/kg and 25.5 ml/min/kg for the males and females, respectively when related to body weight and to values of 1.13 l/min/m² 0.99 l/min/m² when related to BSA. Both the absolute and relative values for $\dot{V}O_{2 \max}$ are lower than normal for Swedish males and females of corresponding ages (12). Individual values were all below -2 S.D. The correlation between $\dot{V}O_{2 \max}$, on the one hand and \dot{Q}_{\max} and SV_{max} on the other was highly significant. No correlation was found with blood volume or HR_{max}.

Mechanical efficiency

$$\left(\frac{\text{mechanical work performed (kpm/min)} \times 100}{427 \cdot 4.9 (\dot{V}O_{2 \text{ work}} - \dot{V}O_{2 \text{ rest}})} \right)$$

was found to be normal in both the male and the female group (1) the mean being $22.9 \pm 1.4\%$ at submaximal load II.

Cardiac output was low when related to $\dot{V}O_{2 \max}$ both during submaximal and maximal work (Fig. 1). Half of the observations were below -2 S.D. of the regression line for young males found by Ekblom et al (12) using techniques similar to those in the

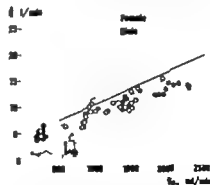


Fig. 1 Cardiac output in relation to oxygen uptake at rest in the supine position and during exercise in the sitting position in 18 totally corrected adult patients with TOF. The regression line ($y = 5.72x + 5.13$ S.D. ± 1.44) is calculated from data of Ekblom et al (12). The present regression equation: $y = 4.56x + 3.8$ S.D. ± 1.39 . Significance of difference between lines 0.05 > p > 0.01.

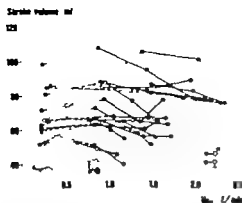


Fig. 2 Individual values for stroke volume in relation to oxygen uptake at rest in the supine position and during exercise in the sitting position in 18 totally corrected adult patients with TOF

present investigation. Only one observation was above this regression line. \dot{Q}_{max} was 13.2 l/min for the males and 10.8 l/min for the females corresponding to cardiac indices of 7.2 and 6.6 l/min/m² for males and females, respectively. No correlation was found between \dot{Q}_{max} and HV blood volume or HR_{max}.

Stroke volumes (SV, ml) at rest in the supine position were small when compared with normal volumes (3.6–28). The mean value for males was 79 ml (range 66–99) and for females 61 ml (range 57–72). Corresponding values for stroke index were 43.4 and 6.6 respectively. SVs at submaximal exercise 1

re slightly but significantly higher ($0.01 < p < 0.001$) than at rest in the supine position. During exercise there was a fall in SV with increasing work loads, mean SV being 76 ml during submaximal exercise I, 70 ml during submaximal exercise II and 67 ml during maximal exercise (Fig. 2). The difference between SV during exercise I and maximal exercise was highly significant ($0.001 < p$). In 8 patients the fall in SV was >10 ml. The fall in SV and the size of SV_{max} could not be correlated to any particular residual lesion. No correlation existed between SV_{max} and blood volume, HV or HR_{max}.

Mean maximal heart rate was 184 ± 9 beats/min for the male group and 179 ± 7 beats/min for the female. HR related to relative work is illustrated in Fig. 3.

Total blood volume was 4.78 ± 1.10 l or 71 ± 15 ml/kg b.wt. in the male group and 4.20 ± 0.43 l or 73 ± 13 ml/kg b.wt. in the female.

Heart volumes were larger than normal. In the male group a mean value of 864 ± 150 ml was found corresponding to 465 ± 36 ml/m² when related to

BSA. In the female subjects the values were 714 ± 136 ml and 439 ± 85 ml/m² respectively.

Values for Hb and lactate concentrations as well as ventilation are summarized in Table III. A detailed statement of spirometric data, pulmonary ventilation, gas exchange and acid-base balance has been given in an earlier publication (7).

DISCUSSION

The values obtained for maximal oxygen uptake are low for both sexes. They are almost identical with the figures found in totally corrected TOF patients by Epstein et al. in a recently published investigation (14) and represent a reduction of 30–40% compared with normal figures (17). These findings show that in spite of a successful anatomic repair, no true normalization of the aerobic work capacity occurs. However, in a study from this department of 16 only shunt-operated TOF patients of comparable age the $\dot{V}O_{2max}$ was found to be only 18.0 ml/kg/min. Thus about 60% higher values were found in the totally corrected subjects. This corresponded well with the subjective evaluation made by the patients themselves who felt that their exercise tolerance had increased considerably after intracardiac repair.

Values for cardiac output related to oxygen uptake were lower than in normal subjects (Fig. 1). This finding is contrary to earlier reports (9, 17, 18, 33, 40).

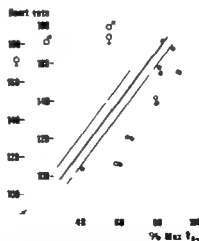


Fig. 3 Individual values for heart rate (beats/min) during submaximal work in the sitting position in relation to oxygen uptake expressed as percentage of the maximal oxygen uptake in 18 totally corrected adult patients with TOF. Regression line with S.D. calculated from data of Astrand (2).

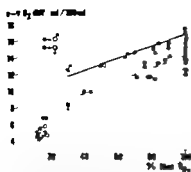


Fig. 4 Arteriovenous oxygen difference in relation to oxygen uptake expressed as percentage of maximal oxygen uptake in 18 totally corrected adult patients with TOF. The regression lines, females $y = 0.07x + 6.40$ and males $y = 0.07x + 9.61$ are calculated from data of Åstrand *et al.* (3). The present regression equations, males $y = 0.08x + 7.11$ and females $y = 0.06x + 7.36$, were not significantly different from those of Åstrand.

which state that a normal increase in \dot{Q} occurred with exercise. In the latter studies however haemodynamic evaluations were performed with the patients working at low exercise loads. The finding of an altered relationship between $\dot{V}O_2$ and \dot{Q} is however in agreement with Epstein's results from 10 corrected patients with TOF without significant residual lesion. The same situation has been found to exist in other types of congenital heart disease: e.g. atrial septal defect, pulmonary valvular stenosis, mitral stenosis, aortic insufficiency (4, 13, 14, 22, 26, 37) both before and after total correction. In the present series no correlation was found between the decrease in \dot{Q} relative to $\dot{V}O_2$ and the type of anatomy in the RV outflow tract, the use of outflow patch or the presence of a particular residual lesion. It is thus likely that the "inadequacy" of the \dot{Q} response to exercise is less related to the type of congenital heart malformation than to the myocardial function itself.

The low \dot{Q} relative to $\dot{V}O_2$ led to a higher-than-normal AV oxygen difference. This does not reflect a more effective oxygen extraction and/or a changed distribution of the left ventricular (LV) output, e.g. from visceral organs to working muscles, since the AV oxygen difference was found to be normal when relative work was used as frame of reference (Fig. 4). Furthermore the AV oxygen difference during maximal exercise expressed as percentage of oxygen binding capacity of the blood was found to be normal (73%).

An analysis of the subfunctions of the cardiac

output, i.e. heart rate and stroke volume, reveals that the main cause of the low \dot{Q} was the small SVs found: 74 ml in the male and 61 ml in the female group during maximal exercise in the sitting position. A highly significant correlation existed between SV_{max} and $\dot{V}O_{2max}$. The reason for the small SVs found cannot be fully elucidated from the results of this study. However, some conclusions could be drawn from the findings in the postoperative catheterizations performed on some of our patients and from previous investigations.

The SV depends not only on the anatomical size of the ventricles, but also on the venous return to the heart, the resistance to emptying of the ventricles and the contractibility characteristics of the heart muscle. Some controversy exists concerning the size of the LV in TOF. Some investigators have found the LV to be normal or slightly small (31, 34) and an underdevelopment of this chamber, severe enough to be of haemodynamic significance is said to be present in less than 1% of the patients (36). However Jamarik *et al.* (23) convincingly showed that cyanotic patients with TOF more than 2 years of age had smaller left HV than normal. With successful shunt operation or total correction an increase in LV end-diastolic volume as well as LV mass occurred to values significantly higher than normal. In spite of this the LV ejection fraction remained subnormal. This latter finding was attributed to a depressed LV function and consequently not to small left HV.

It is well documented that stroke volume is influenced by the degree of physical activity and that it can be increased by physical training (12, 15, 28, 38). Our patients were definitely more inactive than normal during their childhood and they are probably less active physically also after total correction. In the present study only patients stated that they regularly participated in strenuous physical activities. The vast majority of our patients have an occupation which could classify them as white-collar workers. The reduction in SV might therefore theoretically be explained by small circulatory dimensions secondary to prolonged physical inactivity. It has been shown that a high degree of interdependence exists between different components of the oxygen transport system and the degree of physical activity or $\dot{V}O_{2max}$ (20). The correlation matrix (Table III) shows that this was the case in our subjects too, with one exception, namely the dimensions of the cardiovascular system. Though the SV was corre-



Fig. 5 Individual values for stroke volume during maximal exercise in relation to end-diastolic pressure in the right ventricle at rest in 9 subjects with totally corrected TOF.

lated to $V_{O_{2max}}$ this was not the case with blood volume or heart volume. Neither was there any intercorrelation between stroke volume, blood volume and heart size. If the reduction in SV were to be explained by low physical activity alone, one would have expected such correlations to exist. However, it cannot be excluded that physical inactivity was a contributory cause of the reduction of the SVs. It would therefore have been of interest to see whether our patients' SV would have increased after or gained physical training.

One explanation of the small SVs may be an abnormal contractile response of the ventricles and altered compliance characteristics of the ventricular myocardium (14, 23). Several factors such as the myocardial changes in the right ventricle, secondary to the long-standing muscular hypotrophy, non-contractile parts of the myocardium corresponding to the ventriculotomy scar, the patch in the ventricular septum and the RV outflow tract have to be considered as well as the RBBB that is usually present.

An RV outflow tract gradient is usually present even in successfully operated TOF patients (9, 14, 30, 40). This gradient increases during exercise (14, 40). To maintain an adequate SV in this situation there are three possibilities: to increase the RV systolic pressure, to prolong the ejection time and to increase the diastolic filling (22). Each of these compensatory mechanisms has been found in TOF (9, 14, 23, 40). However, SV decreased during exercise in the present group. If the RV is the limiting factor for maintenance of the SV, this indicates that the above mentioned mechanisms are not sufficient. At

higher HR, a longer duration of systole results in a shorter diastole. This may hamper ventricular filling when an increased filling pressure is needed and the ejection time is prolonged. One may speculate whether this causes the fall in SV during exercise in some of our patients. Support for this theory may be the observed negative correlation between the end-diastolic pressure in RV and the size of SV_{max} ($0.01 \leq p < 0.001$) and SV/BSA ($0.05 \leq p < 0.01$) found in our series (Fig. 5). However, this latter finding must be viewed with caution due to the small number of observations and the fact that data of end-diastolic pressure and SV were obtained on different occasions.

The slightly low maximal heart rate agrees with findings of Hurvitz and Goldberg (21) that the HR_{max} was lower than normal not only before, but also after operative correction of different cardiac lesions. Similar or related observations have been reported by others (16, 26, 27, 42). The reason for the subnormal HR_{max} in cardiac patients is not fully understood. One possible explanation is that the subjects stop exercising for other reasons than circulatory such as e.g. dyspnoea or hypoxaemia (26). This might be the explanation in uncorrected but hardly in totally corrected TOF patients since their ventilatory response to exercise is normal and since they do not have hypoxaemia (7). Another explanation may be that the lower maximal cardiac frequency represents a physiological adaptation to the haemodynamic situation. A lower-than-normal heart rate may be needed to allow optimal diastolic filling. If this explanation is valid, one would expect not only the HR_{max} to be lower than normal, but also the HR at submaximal loads. That this is the case is shown in Fig. 3. Other possible explanations may be related to muscular ventricular hypertrophy or to the presence of conduction disturbances, e.g. RBBB. It should however be noted that in the series of Hurvitz and Goldberg (21) patients with conduction disturbances were excluded.

In the present study the decrease in aerobic capacity could not be correlated to the anatomy of the RV outflow tract or to the use of an outflow patch. Though the number of patients studied was small, this tends to substantiate the findings of others (9, 18, 24, 33) that TOF patients tolerate PI reasonably well. This seems to be the case also when correction is undertaken at adult age.

There was no complete normalization of physical

work capacity in our patients the majority of whom were corrected at adult age. Future studies will show whether a better normalization is achieved if total correction is undertaken in early life. This might perhaps be expected if the TOF patients perform a normal degree of physical activity during the years when growth of circulatory dimensions takes place. The reduction in $\dot{V}O_{2\max}$ was moderate and did not interfere with everyday activities. However it means that at a certain degree of activity our subjects utilized a greater portion of their aerobic work capacity. A greater portion of the cardiac reserve was mobilized. This may prove to be a disadvantage as the patients grow older and are subjected to the ageing processes in the heart and to ischaemic heart disease.

ACKNOWLEDGEMENT

This study was supported by grant from the Swedish National Association against Heart and Chest Diseases.

REFERENCES

- 1 Åstrand, I. Aerobic work capacity in men and women with special reference to age. *Acta physiol. scand. Suppl.* 169 1966.
- 2 Åstrand, P-O. Experimental studies of physical working capacity in relation to sex and age. Munksgaard Copenhagen 1952.
- 3 Åstrand, P-O, Cuddy T E, Saltin, B. & Steinberg J. Cardiac output during submaximal and maximal work. *J appl Physiol.* 19: 268 1964.
- 4 Åström, H. Effect of posture on circulation and respiration at rest and during exercise in heart disease. *Acta physiol. scand. Suppl.* 347 1970.
- 5 Aperia, A, Björke, B, Broberger O & Thorén C. Renal function in Fallot's tetralogy. *Acta paediat. scand.* 63: 398 1974.
- 6 Bevingård, S., Holmgren A. & Jonsson B. The effect of body position on the circulation at rest and during exercise with special reference to the influence on the stroke volume. *Acta physiol. scand.* 49: 279 1960.
- 7 Björke B. Spirometric data, pulmonary ventilation and gas exchange at rest and during exercise in adult patients with tetralogy of Fallot. *Scand. J. resp. Dis.* 55: 47 1974.
- 8 Björke B, Eriksson, B O & Sahlin, B. ATP CP and lactate concentrations in muscle tissue during exercise in male patients with tetralogy of Fallot. *Scand. J. clin. Lab. Invest.* 33: 255 1974.
- 9 Brissow D, Kloster F, Löw, H, Mennarhe, V, Grifvold, H & Starr A. Serial cardiac catheterizations and exercise hemodynamics after correction of tetralogy of Fallot. *Circulation* 41: 1057 1970.
- 10 Burnell, R. H, Woodson, D, Lees, M. H, Brissow J D & Starr A. Result of correction of tetralogy of Fallot in children under four years of age. *J thorac. cardiovasc. Surg.* 57: 153 1969.
- 11 Cramp, D O. Automated enzymatic fluorometric method for the determination of pyruvic and lactic acids in blood. *J. clin. Path.* 21: 171 1968.
- 12 Ekblom, B, Åstrand P-O, Saltin, B, Steinberg, J & Wallström B. Effect of training on circulatory response to exercise. *J appl Physiol.* 24: 518, 1968.
- 13 Engloff E. Aortic incompetence. *Acta med. scand., Suppl.* 538 1972.
- 14 Epstein S, Benzer D, Goldstein, R, Roseng M, Radwood D. & Morrow A. Hemodynamic abnormalities in response to mild and intense upright exercise following operative correction of an atrial septal defect or tetralogy of Fallot. *Circulation* 47: 1065 1973.
- 15 Eriksson B O & Koch, G. Effect of physical training on hemodynamic response during submaximal and maximal exercise. 11-13 year old boys. *Acta physiol. scand.* 87: 27 1973.
- 16 Goldberg, S J, Weiss, R. & Adams, F H. A comparison of the maximal endowments of normal children and patients with congenital cardiac disease. *Pediatrics* 69: 46, 1966.
- 17 Götteman M. S. Haemodynamic and cine angiocardiographic findings after one stage repair of Fallot tetralogy. *Brit. Heart J.* 28: 448 1966.
- 18 Götteman, M S, Beck, W, Bernard C, N. O, Donovan, T O & Schrire V. Results of repair of tetralogy of Fallot. *Circulation* 40: 803 1969.
- 19 Hare, A., Rastelli, G C, Rytter D O, DuShane, J W & McGoon, D C. Management of the right ventricular outflow tract in severe tetralogy of Fallot. *J thorac. cardiovasc. Surg.* 60: 131 1970.
- 20 Holmgren A. & Åstrand P-O. O_2 and the diastolic and functional capacities of the O_2 transport system in humans. *J appl Physiol.* 21: 1463 1966.
- 21 Horvitz, R. & Goldberg S. Maximal cardiac rate before and following repair of cardiac lesions. *J Sport Med.* 10: 163 1970.
- 22 Iklos, D, Jonsson B & Linderholme, H. Effect of exercise in pulmonary stenosis with intact ventricular septum. *Brit. Heart J.* 28: 316 1966.
- 23 Jarnheden J, Graham T J, Canest, R. & Jewett, P. Left heart function in children with tetralogy of Fallot before and after palliative or corrective surgery. *Circulation* 46: 478, 1972.
- 24 Jones, E L, Cori, R. C, Neill, C A, Gott, V L, Brawley R. K. & Haller A J. Long-term evaluation of tetralogy patients with pulmonary valvular insufficiency resulting from outflow-patch correction across the pulmonary annulus. *Circulation, Suppl.* 3: 11 1973.
- 25 Jonasson, S. A method for the determination of the heart size by teleoroentgenography. *Acta radiol.* 20: 325 1939.
- 26 Jonsson, B. Circulatory adaptation to exercise in congenital heart disease. *Proc. Ann. Europ. Paediat. Cardiol.* 9: 2, 1973.
- 27 José, A. D. & Taylor R. R. Autonomic blockade by propranolol and atropine to study intrinsic myocardial function in man. *J. clin. Invest.* 43: 2019 1969.
- 28 Kilbom, Å & Åstrand I. Physical training with submaximal intensities in women. II Effect on cardiac output. *Scand. J. clin. Lab. Invest.* 28: 143 1971.

29. Klusman J M, Moore J W & Hamilton, W F. Studies on the circulation. I Injection method. Physical and mathematical considerations. *Amer J Physiol* 99: 322, 1929
30. Kirkila, J & Karp R. The tetralogy of Fallot. Saunders, Toronto 1970
31. Lew M, Rimoldi H J A & Rowlett U. The quantitative anatomy of cyanotic tetralogy of Fallot. *Circulation* 30: 531 1964
32. Lillehei, C. W. Cohen M, Warden, H. E. Read R. C., Aust, J II, DeWall, R. A & Varco R. L.. Direct vision intracardiac surgical correction of the tetralogy of Fallot, pentalogy of Fallot and pulmonary atresia defects: report of first ten cases. *Ann Surg.* 142: 418, 1955
33. Malin, J R. Blumenthal S., Bowman, F O, Ellis, K., Jamerson, A. G, Jenne M J & Yeoh C B. Factors that modify hemodynamic results in total correction of tetralogy of Fallot. *J thorac cardiovascular Surg* 52: 502, 1966
34. Miller O, Kirklin J, Rahlemoola S & Swan H. Volume of left ventricle in tetralogy of Fallot. *Amer J Cardiol*, 18, 488 1965
35. Möller T. Shunt operations in morbus Caeruleus. *Acta paediat scand* Suppl. 134 196..
36. Nagao G. I. Daoud, O. I. McAdams, A. J. Schwartz, D. C. & Kaplan S. Cardiovascular anomalies associated with tetralogy of Fallot. *Amer J Cardiol* 20: 706 1967
37. Peterson, E. O. Atrial septal defect of secundum type: A clinical study before and after operation with special reference to haemodynamic function. *Acta paediat. scand.*, Suppl. 174 1967
38. Sakon, B., Blomqvist, G, Mitchell J H, Johnson, R. L., Jr, Wibbenhal, K. & Chapman B. Response to exercise after bed rest and after training. *Circulation*, Suppl. 7 1968
39. Scholander T F. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J Biol Chem.* 167 235 1947
40. Shah, P. & Kidd, L. Hemodynamic responses to exercise and to isoproterenol following total correction of Fallot tetralogy. *J thorac cardiovasc. Surg.* 52: 138, 1966.
41. Snedecor G W. Statistical methods 6th ed. The Iowa State University Press Iowa 1967
42. Varner S & Braunwald E. Cardiac frequency control and adjustments to alterations. *Progr cardiovasc Dis.* 14 431 1972.
43. Williams J A. & Fine, J. Measurement of blood volume with a new apparatus. *New Engl. J Med.* 264 842, 1961

OXYGEN UPTAKE, ARTERIAL BLOOD GASES AND BLOOD LACTATE CONCENTRATION DURING SUBMAXIMAL AND MAXIMAL EXERCISE IN ADULT SUBJECTS WITH SHUNT-OPERATED TETRALOGY OF FALLOT

Bengt O Eriksson and Björn Björke

*From the Department of Pediatrics, Karolinska Institute,
St. Göran Children Hospital Stockholm, Sweden*

Abstract. Ten female and six male adult subjects with shunt-operated tetralogy of Fallot have been studied at rest and during submaximal and maximal exercise on an average 70 years after the palliative operation. There was considerable reduction in the aerobic work capacity, maximal oxygen uptake ($\dot{V}O_2$) being 1.00 l/min STPD. Though ventilation (\dot{V}_E) was out of proportion to $\dot{V}O_2$ as indicated by an abnormally high ventilation equivalent ($\dot{V}_E/\dot{V}O_2$), values for $\dot{V}_{E_{max}}$ were low (50.8 l/min BTPS) and of approximately the same order as in a comparable group of totally corrected TOF patients. In spite of increased \dot{V}_E in relation to $\dot{V}O_2$, the P_{CO_2} increased from 30 mmHg at rest to 49 mmHg during maximal exercise, while P_{O_2} decreased from 60 mmHg at rest to 44 mmHg during submaximal exercise I. During heavier exercise no further fall was noted. Base excess decreased from -2.9 to -9.8 mEq/l. Thus combined respiratory and metabolic acidosis was at hand during exercise, the metabolic component, however being normal. Maximal blood lactate concentrations were low (5.8 mmol/l) and contrasted with the high intramuscular lactate concentrations earlier reported in some of the patients. The low values found for $\dot{V}O_{2_{max}}$ indicate that palliative operative procedure in TOF is no alternative to an intracardiac repair in the long-term course. The two main factors limiting exercise tolerance were acidosis and accumulation of lactate within the muscle cell.

Tetralogy of Fallot (TOF) is characterized by an obstruction of the outflow tract of the right ventricle in combination with a non-pressure limiting ventricular septal defect, enabling shunting of the blood from either the left ventricle to the right or vice versa. Before the time when palliative shunt operations were introduced the prognosis for TOF was fairly bad (74). The determining factor for the prognosis in the individual subject was the degree of reduction of the blood flow to the lungs. Therefore

the aim of the first operations in TOF was to increase the pulmonary blood flow (7). These operations though only palliative improved the prognosis (21).

Reports on the long-term prognosis in palliated TOF are now available (8, 15, 23, 29). However data concerning the aerobic working capacity and reaction to exercise are few in adult subjects with TOF. Still an anticipated reduced working capacity is used as an argument in fa- our of total correction. The strength of this particular argument, however depends on how severe the reduction is and how handicapped palliated patients are in their activities of daily life.

Thus the aim of this study was to examine subjects with palliated TOF who had lived for rather a long time after their shunt operation, in respect of the following questions:

1. The adaptation of ventilation, oxygen uptake and gas exchange during submaximal and maximal exercise.
2. The effect of hypoxaemia during exercise on the anaerobic metabolism as judged from the oxygen deficit and the blood lactate concentration.

SUBJECTS

Six male and ten female subjects, aged 22-35 years, were studied. They all belonged to the first shunt-operated group of TOF patients in Sweden. Data from follow-up 5.5 years after the shunt-operation have earlier been published by Möller (21). Of the 30 subjects who had not been subjected to an intracardiac repair 1 were still alive from whom 16 subjects were recruited all having Blalock-Taussig anastomosis. As the general opinion is and has for several years been that patients with TOF should have an intracardiac repair performed around the age of 5 years (20), and as 20 years had elapsed since the original shunt opera-

Present address: Department of Pediatrics, University of Göteborg, Östra Sjukhuset, S-416 85 Göteborg, Sweden

- 29 Kinsman J M Moore J W & Hamilton W F. Studies on the circulation. I Injection method. Physical and mathematical considerations. *Amer J Physiol* 89: 322, 1929
- 30 Kirklin J & Karp R. The tetralogy of Fallot. Saunders, Toronto 1970.
- 31 Lew M Rimoldi H J A. & Rowlett U. The quantitative anatomy of cyanotic tetralogy of Fallot. *Circulation* 30: 531, 1964
- 32 Lillehei C W Cohen H Warden H E Read R C Aust J B DeWall R A & Varco R L. Direct vision intracardiac surgical correction of the tetralogy of Fallot: pentalogy of Fallot and pulmonary atresia defects: report of first ten cases. *Ann. Surg.* 142: 418, 1955
- 33 Malin J R Blumenthal S. Bowman, F O Ellis, K. Jameson, A. G Jesse M. J & Yeoh, C B. Factors that modify hemodynamic results in total correction of tetralogy of Fallot. *J thorac. cardiovasc. Surg.* 52: 502, 1966
- 34 Miller H Kirklin J Rahimtoola, S & Swan, H. Volume of left ventricle in tetralogy of Fallot. *Amer J Cardiol* 16: 488, 1965
- 35 Möller T. Shortest operations in morbus Caeruleus. *Acta paediat. scand. Suppl.* 134, 1962.
- 36 Nagao G I Daoud, G I McAdams, A. J Schwartz, D C. & Kaplan S. Cardiovascular anomalies associated with tetralogy of Fallot. *Amer J Cardiol* 20: 206, 1967
- 37 Peterson, H O. Atrial septal defect of secundum type: A clinical study before and after operation with special reference to haemodynamic function. *Acta paediat. scand. Suppl.* 174, 1967
- 38 Saltin B., Blomqvist, G Mitchell, J H., Johnson, R. L. Jr Widenthal K. & Chapman H. Response to exercise after bed rest and after training. *Circulation, Suppl.* 7, 1968
- 39 Scholander P F. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J. biol. Chem.* 167: 235, 1947
- 40 Shah P & Kidd L. Hemodynamic responses to exercise and to isoproterenol following total correction of Fallot's tetralogy. *J. thorac. cardiovasc. Surg.* 52: 138, 1966.
- 41 Snedecor G W., *Statistical methods*, 6th ed. The Iowa State University Press Iowa 1967
- 42 Vetter S & Braunwald E. Cardiac frequency control and adjustments to alterations. *Progr. cardiovasc. Dis.* 14: 431, 1972.
- 43 Williams, J A. & Fine J. Measurement of blood volume with new apparatus. *New Engl. J. Med.* 264: 842, 1961

Table II. Mean values \pm S.D. for ventilation, oxygen uptake, oxygen deficit and heart rate during submaximal and maximal exercise in shunt-operated TOF

No. of subjects given within parentheses

	Submax. I (221 kpm/min)	Submax. II (373 kpm/min)	Max. exercise (516 kpm/min)
\dot{V}_E (l/min BTPS)	23.7 \pm 8.3 (14)	36.7 \pm 8.9 (13)	30.8 \pm 18.6 (16)
$\dot{V}O_2$ (l/min STPD)	0.65 \pm 0.15 (14)	0.90 \pm 0.19 (13)	1.00 \pm 0.30 (16)
$\dot{V}_E/\dot{V}O_2$	36.6 \pm 10.5 (14)	42.5 \pm 12.8 (13)	52.1 \pm 17.5 (16)
R	0.80 \pm 0.08 (14)	0.92 \pm 0.08 (13)	1.00 \pm 0.09 (16)
Oxygen deficit (l)	0.60 \pm 0.33 (13)	1.16 \pm 0.43 (13)	1.83 \pm 0.56 (16)
HR (beats/min)	118 \pm 16 (14)	143 \pm 19 (13)	167 \pm 13 (16)

Clark type microelectrode with polypropylene membrane, P_aCO_2 and base excess were obtained from a curve nomogram, the values being corrected for low arterial saturation when present (27). The reproducibility of the methods in our laboratory has been reported elsewhere (13). Hb concentration was measured spectrophotometrically (16) and lactate concentration enzymatically (10).

Calculations

Oxygen deficit was calculated assuming a normal mechanical efficiency of 11% (1, 12). The difference between the $\dot{V}O_2$ calculated from the assumed mechanical efficiency and the total measured $\dot{V}O_2$ during the whole work period of 4 min was taken as the oxygen deficit at that particular load. In the two subjects whose work time was 6 min, the first

portion of expired air was collected during the first 3 min and the second portion during the last 2 min of exercise. Oxygen deficit in these two subjects was calculated according to Karlsson (19).

Comments

Four-minute exercise periods were chosen as it has been shown that during work of longer duration an i.m. utilization of lactate takes place in normal subjects (18). Thus blood lactate concentration in longer exercise periods does not fully reflect the anaerobic metabolism. However, short work periods mean that values for $\dot{V}O_2$ during submaximal exercise are not quite comparable with those for longer exercise periods. This was one factor which made calculations of mechanical efficiency in our subjects impossible.

Table III. Mean values \pm S.D. for arterial blood gases, haemoglobin and blood lactate concentrations at rest and during exercise in shunt-operated TOF

No. of subjects given within parentheses

	Rest	Submax. I (221 kpm/min)	Submax. II (373 kpm/min)	Max. exercise (516 kpm/min)
P_aO_2 (mmHg)	60 \pm 8.0 (13)	44 \pm 8.6 (10)	45 \pm 5.0 (9)	42 \pm 7.1 (13)
P_aCO_2 (mmHg)	30 \pm 4.0 (13)	42 \pm 9.9 (10)	41 \pm 7.8 (9)	49 \pm 13.8 (13)
pH	7.43 \pm 0.02 (13)	7.32 \pm 0.06 (10)	7.30 \pm 0.06 (9)	7.22 \pm 0.06 (13)
Base excess (mEq/l)	-2.9 \pm 1.9 (13)	-5.1 \pm 1.5 (10)	-6.9 \pm 2.5 (9)	-9.8 \pm 2.9 (13)
Hb concentration (g/100 ml)	18.2 \pm 4.7 (16)	19.0 \pm 5.0 (7)	18.8 \pm 5.0 (8)	18.6 \pm 5.2 (11)
Blood lactate concentration (mmol/l)	0.8 \pm 0.3 (16)	2.2 \pm 0.6 (11)	3.3 \pm 0.9 (11)	5.8 \pm 1.5 (15)

RESULTS

The ten female and six male subjects worked at two submaximal loads beside the maximal one. The latter averaged 450 kpm/min for the female 625 kpm/min for the male subjects and 516 kpm/min for the total group. The work times at maximal exercise loads were 3.9, 3.7 and 3.8 min respectively. Mean values of the data obtained during exercise are given in Tables II and III. Oxygen uptake was 0.65 l/min and 0.90 l/min at submaximal load I (221 kpm/min) and submaximal load II (373 kpm/min) respectively. During maximal exercise a value of 1.00 l/min was obtained. Total ventilation increased from 23.7 l/min during submaximal I to 50.8 l/min during maximal exercise (Fig. 1) giving a ventilation equivalent of 52.1 during maximal exercise.

Oxygen deficit was 0.60 l during submaximal I, 1.16 l during submaximal II and 1.83 l during maximal exercise. Heart rate increased from a resting value of 77 to 167 beats/min during maximal exercise. P_{O_2} was 60 mmHg at rest. During exercise it decreased to 44 mmHg at the lowest work load, but no further fall occurred at higher work loads. P_{CO_2} was 30 mmHg at rest and increased during exercise to 42, 41 and 49 mmHg (Fig. 2). The increase between submaximal I and maximal exercise is statistically significant ($p < 0.05$). Base excess dropped from -2.9 to -9.8 mEq/l during maximal exercise and arterial pH from 7.43 to 7.22 (Fig. 2). Arterial blood lactate concentration during maximal exercise was 5.8 mmol/l. Arterial Hb concentration was around 19 g/100 ml both at rest and during exercise.

COMMENTS

In this study maximal oxygen uptake was defined as the value for oxygen uptake found at the heaviest load on which the subjects could work for 4 min according to information obtained at the preliminary test. The very high values for V_E/V_{O_2} and R support the subjective impression of the investigator that the patients worked at or near their maximum. Thus the obtained values for V_{O_2} at maximal work loads could be looked upon as acceptable for $V_{O_{2max}}$.

DISCUSSION

The few reports published on aerobic working capacity in subjects with TOF have shown low values (11, 12, 14, 28, 30). However the reported materials differ from ours. In Taylor's material (30) there were only 9 of 16 reported subjects with non-

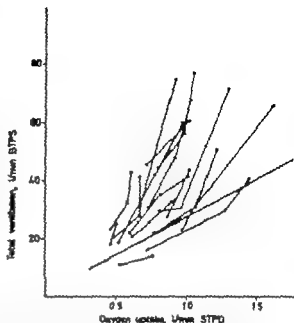


Fig. 1 Individual values for total ventilation in relation to oxygen uptake at submaximal and maximal exercise loads in subjects with shunt-operated TOF. Solid line denotes regression line for normal subjects (4).

totally corrected TOF and most of them were acyanotic. In the material of Gold et al. (14) all 17 subjects studied had palliated TOF but in most of them the shunt was a Potts anastomosis. Also the material of Davies and Gazetopoulos (12) differs from ours. Of their 20 subjects 13 had TOF but only 5 of them had had a shunt operation performed. In the material of Crawford et al. (11) the 21 TOF subjects were all palliated, 13 with a Blalock-Taussig anastomosis and the rest with Potts anastomosis (22); the latter however having an increased pulmonary blood flow. Thus our results are not quite comparable with those reported by others. On the other hand our series has the longest follow-up and is uniform as in all cases the anastomosis performed was of the Blalock-Taussig type (7). Therefore our values for working capacity add information about the situation in adult shunt-operated subjects with TOF.

$V_{O_{2max}}$ was around 30% of the normal in Sweden (1). The six male subjects had slightly higher values, 1.21 l/min and 22.2 ml/kg/min, than the female subjects, 0.88 l/min and 15.5 ml/kg/min, in spite of a deeper cyanosis and higher Hb concentration (Table I). Even if the difference in body composition is considered (2), higher values for the aerobic power were found in the male subjects. The reason for this

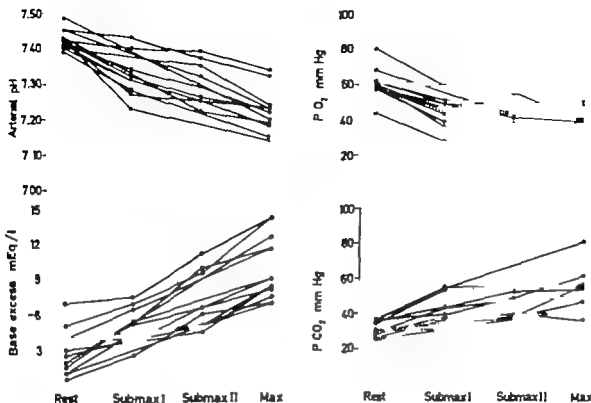


Fig. 2 Individual values for arterial oxygen tension ($P O_2$), arterial carbon dioxide tension ($P CO_2$), arterial pH and

base excess at rest, at submaximal and maximal exercise load in subjects with shunt-operated TOF

is obscure. However, all six male subjects were physically active in their daily work, while many of the females were relatively inactive. Thus the degree of cyanosis as reflected in the arterial saturation at rest and in the Hb concentration do not always give information about the degree of lowered working capacity.

Many activities of daily life require a $\dot{V} O_2$ around or above 1 l/min (2). This means that shunt-operated adult subjects with TOF are easily forced to a working level near the maximal. If therefore aerobic power is used as a criterion of these subjects' situation it speaks strongly in favour of an intracardiac repair. However, many of the subjects stated that they experienced no or only mild discomfort during daily life. The same observation was made by Gold et al. (14). This emphasizes that subjective information about exercise tolerance from a subject with TOF must be looked upon with certain degree of caution.

In another study (4) the reduced values for vital capacity and total lung capacity in these subjects as well as in a comparable group of totally corrected

TOF patients were reported. It was also found that the totally corrected patients had a normal ventilatory response to exercise. Though $\dot{V} E_{max}$ was low (61.9 l/min) it matched the $\dot{V} O_{2max}$, giving normal $\dot{V} E/\dot{V} O_2$ ratio and did not constitute a limiting factor for working capacity. In the present study a slightly lower value for $\dot{V} E_{max}$ was obtained (50.8 l/min) in spite of the roughly 40% lower $\dot{V} O_{2max}$. This resulted in a very high $\dot{V} E/\dot{V} O_2$ ratio during maximal exercise. This should not be taken as an absolute indication, however, that excessive $\dot{V} E$ or increased respiratory work limits the working capacity in palliated TOF patients as their $\dot{V} E_{max}$ is if anything lower than in the comparable group of totally corrected subjects.

An abnormal $\dot{V} E/\dot{V} O_2$ ratio was present also during submaximal exercise (Fig. 1) and possibly also at rest as judged by the low $P CO_2$. The hyperventilation present at rest cannot be fully explained by the experimental situation (1) as the $P CO_2$ was lower than in totally corrected TOF subjects in the same situation (4). The subjects hyperventilated in spite of

normal pH and hypocapnia, leaving hypoxia as a possible stimulus to ventilation at rest.

It is obvious that part of the ventilations is "wasted" in cyanotic patients with TOF as blood is shunted past the lungs at cardiac level. An increase in dead space ventilation within the lung in palliated TOF patients has been reported (14-28) and should, if present, contribute to the wasted ventilation. The finding in the present series of a P_{CO_2} of 30 mmHg at rest must imply, in the presence of an intracardiac right-to-left shunt, a still lower PCO_2 in the pulmonary veins indicating an alveolar hyperventilation. Whether the increased total ventilation leads to a uniform proportional increase in dead space ventilation and alveolar ventilation during exercise is not known. The main component of the wasted ventilation, however, is most likely secondary to the increasing right-to-left intracardiac shunt during exercise and not to an increase in the physiological dead space within the lung.

When changing from rest in the supine position to exercise in the sitting position an increase in P_{CO_2} was found. Even slightly higher values were obtained during maximal exercise. The possibility that the altered body position in itself may partly explain the results cannot be excluded. However, shunting at the cardiac level of venous blood with an increased CO_2 tension during exercise seems to be the main reason for the values found. The subjects were unable to compensate for the normal metabolic costs occurring during exercise with a respiratory sin as a respiratory acidosis existed in spite of high $VE/\dot{V}O_2$ ratio.

A fall in P_{O_2} occurred during exercise due to an increasing intracardiac right-to-left shunt (20). However, during heavier exercise no further fall in P_{O_2} was noted. This may partly be explained by an increasing pulmonary blood flow due to left-to-right shunts through aortopulmonary collateral circulation and via the Blalock-Taussig anastomosis. The flow through these anastomoses increases during heavy exercise since the mean pressure in the aorta increases while the pressure in the pulmonary arteries remains unchanged. However, in the presence of an increasing P_{CO_2} , a corresponding decrease in S_{O_2} must take place. The unchanged P_{O_2} must therefore partly be attributed to a right displacement of the oxyhaemoglobin dissociation curve secondary to increasing acidosis.

Judged by the base excess value obtained during maximal exercise the metabolic component of the

acidosis was not abnormally pronounced. However, due to the inability to eliminate CO_2 , a respiratory component was added to the acidosis resulting in subnormal pH values.

Blood lactate concentration was normal at rest. During exercise an increase was found but values during maximal exercise were considerably lower than normal. A subnormal blood lactate response to exercise in patients with cyanotic heart diseases has been found by others (28). This has formed the basis for the statement that these patients stop working for other reasons than muscular, or that no hypoxaemia incapacitated the muscle (28). In the present series the normal metabolic component of the acidosis, subnormal blood lactate values and the finding of similar P_{O_2} values during exercise of different intensities tend to substantiate this statement. However, in the exercising muscle in those of our patients investigated the lactate concentration was found to be high (6). This was attributed to circulatory factors and inability to eliminate lactate from the muscle cell. This means that the blood lactate concentration does not fully reflect the anaerobic metabolism during exercise in subjects with TOF.

Heart rate at maximal exercise was 167 beats/min. This "maximal heart rate" is almost 30 beats lower than what is considered normal (1-2). Whether or not this is due to a real lower maximal heart rate is difficult to say. It is possible that the subjects reach the exercise breaking point before the maximal heart rate potential is reached. On the other hand studies on comparable subjects with TOF who had had an intracardiac repair show that they attain only a slightly higher maximal heart rate (5).

From the above discussion it is concluded that the two main factors limiting exercise tolerance were acidosis and accumulation of lactate within the muscle cell. Both are related to circulatory factors, the acidosis being due to CO_2 accumulation secondary to low pulmonary blood flow and the lactate accumulation possibly to altered blood flow to the muscle.

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish National Association against Heart and Chest Diseases.

REFERENCES

1. Åstrand I. Aerobic work capacity in men and women with special reference to age. *Acta physiol scand.* Suppl. 169. 1960.

2. Åstrand, P-O & Rodahl, K. Textbook of work physiology McGraw Hill New York 1970
3. Aperia, A, Björke, B, Broberger, O & Thoren, C. Renal function in Fallot tetralogy. *Acta paediat. scand.* 63: 398 1974
4. Björke, B. Spirometric data, pulmonary ventilation and gas exchange at rest and during exercise in adult patients with tetralogy of Fallot. *Scand. J. resp. Dis.* 55: 47 1974
5. —. Oxygen uptake and cardiac output during submaximal and maximal exercise in adults with totally corrected tetralogy of Fallot. *Acta med. scand.* 197: 177 1975
6. Björke, B, Eriksson, B O & Sævi, B. ATP, CP and lactate concentrations in muscle tissue during exercise in adult patients with tetralogy of Fallot. *Scand. J. clin. Lab. Invest.* 33: 225 1974
7. Blalock, A. & Taussig, H. B. The surgical treatment of malformations of the heart in which there is pulmonary stenosis or pulmonary atresia. *J.A.M.A.* 128: 189 1945
8. Cole, R. B., Mieser, A. J., Flader, D. E. & Paul, M. H. Long-term results of aortopulmonary anastomosis for tetralogy of Fallot. *Circulation* 43: 263 1971
9. Connor, J. H., J. Forrester, R. E., Debois, A. B., Briscoe, W. A. & Carlsen, E. The lung. Year Book Publishers, Chicago 1967
10. Crump, D. G. Automated enzymatic fluorometric method for the determination of pyruvic and lactic acids in blood. *J. clin. Path.* 21: 171 1968
11. Crawford, D. W., Simpson, E. & McIlroy, M. B. Cardiopulmonary function in Fallot tetralogy after palliative shunting operations. *Amer. Heart J.* 74: 463 1967
12. Davies, H. & Gazetopoulos, N. Dyspnoea in cyanotic congenital heart disease. *Brit. Heart J.* 77: 28 1965
13. Eriksson, B O. Physical training, oxygen supply and muscle metabolism in 11-13 year old boys. *Acta physiol. scand. Suppl.* 384 1972
14. Gold, W. M., Matzok, L. F. & Price, A. C. Response to exercise in patients with tetralogy of Fallot with systemic-pulmonary anastomoses. *Pediatrics* 43: 781 1969
15. Harris, A. M., Segal, H. & Bishop, J. M. Blalock-Taussig anastomosis for tetralogy of Fallot. A ten- to fifteen-year follow-up. *Brit. Heart J.* 26: 266 1964
16. Holmgren, A. & Pernow, B. Spectrophotometric measurement of oxygen saturation of blood in the determination of cardiac output. A comparison with the van Slyke method. *Scand. J. clin. Lab. Invest.* 11: 143 1959
17. Jonsell, S. A method for determination of the heart size by teleoroentgenography. *Acta radiol.* 70: 395 1939
18. Jorfeldt, L. Metabolism of L(+)-lactate in human skeletal muscle during exercise. *Acta physiol. scand. Suppl.* 338 1970
19. Karlsson, J. Lactate and phosphagen concentrations in working muscle of man. *Acta physiol. scand., Suppl.* 338 1971
20. Kirklin, J. W. & Karp, R. B. The tetralogy of Fallot. Saunders Toronto 1970
21. Möller, T. Shunt operations in morbus caeruleus. *Acta paediat. scand. Suppl.* 134 1962
22. Potter, W. J., Smith, S. & Gibson, S. Anastomosis of the aorta to a pulmonary artery in certain types of congenital heart disease. *J. A.M.A.* 132: 631 1946
23. Rygg, I. H., Berthelsen, S., Borgesen, S., Faberius, J., Fritz Hansen, P., Høivær, E., Lærudsen, P., Melchior, J. & Sande, E. The palliative surgical treatment of tetralogy of Fallot. In: Tetralogy of Fallot. A study of all cases diagnosed in Denmark from 1947 to 1965. *Dan. med. Bull. Suppl.* 2: 99 1971
24. Rygg, I. H., Olsson, K. & Boesen, I. The history of tetralogy of Fallot. In: Tetralogy of Fallot. A study of all cases diagnosed in Denmark from 1947 to 1965. *Dan. med. Bull. Suppl.* 2: 23 1971
25. Scholander, P. F. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J. biol. Chem.* 167: 235 1947
26. Shephard, R. J. The resting hyperventilation of congenital heart disease. *Brit. Heart J.* 17: 13 1955
27. Seegard-Andersen, O., Engel, K., Jørgensen, K. & Astrup, P. A micro-method for determination of pH, carbon dioxide tension, base excess and standard bicarbonate in capillary blood. *Scand. J. clin. Lab. Invest.* 12: 172 1960
28. Strieder, D. J., Mark, Z. G., Zawer, A. G. & Gold, W. Exercise tolerance in chronic hypoxemia due to right-to-left shunt. *J. appl. Physiol.* 34: 873 1973
29. Tau, G., H. B. Crocetti, A., Eschepower, E., Kemonen, R., Yap, M., Bachman, D., Mombberger, N. & Kirk, H. Fifteen year follow-up on the first six years of the Blalock-Taussig operation. In: The natural history and progress in treatment of congenital heart defect (ed. L. Kadd and J. D. Keith) p. 83. Thomas Springfield, Illinois 1971
30. Tylor, M. R. H. The ventilatory response to hypoxia during exercise in cyanotic congenital heart disease. *Clin. Sci.* 45: 99 1973

HAEMODYNAMIC EFFECT OF ATRIAL TRIGGERED VERSUS FIXED RATE PACING AT REST AND DURING EXERCISE IN COMPLETE HEART BLOCK

Ingvar Karlöf

*From the Department of Clinical Physiology Thoracic Clinics
Karolinska H. spinal, Stockholm Sweden*

Abstract The central haemodynamics at rest and during exercise have been studied in 25 patients with complete AV block who were treated with fixed rate (FRP) and atrial triggered pacemakers (ATP). The aim of the investigation has been to study the effect of synchronized atrial contraction for the filling of the ventricles and for the cardiac output (Q). Pressures and Q have been determined during least catheterization. The P wave for triggering the atrial synchronized pacemaker has been obtained with an electrode in close contact with the atria, introduced by means of mediastinoscopy. The study consists of two series. In the first series (12 patients) the central haemodynamics were recorded with each patient connected first the FRP (about 70 beats/min) and later to the ATP. Most patients were studied both at rest and during exercise, the work loads being identical with both types of pacemakers. Q at rest is 10% higher with ATP ($p < 0.02$) than with FRP and during exercise 20% higher with ATP ($p < 0.01$). Stroke volume (SV) at rest is equal with both types of pacemakers, but significantly larger with FRP during work ($p < 0.001$). The left ventricular (LV) filling pressure is significantly lower ($p < 0.01$) with ATP at rest, but not during exercise ($p < 0.5$). In the second series 13 patients were studied at rest and during exercise. The recordings of pressures and Q were first performed with the patient on ATP. After 30 min rest an identical study was performed with the patient connected to FRP, the rate of which was matched (FRPa) to that previously recorded with ATP. Q at rest is 18% higher ($p < 0.01$) with ATP than with FRPa and during work 8% higher ($p < 0.05$) with ATP. SV at rest is significantly larger ($p < 0.01$) with ATP than with FRPa, whereas during exercise no significant difference is observed between the two types of pacemakers. LV filling pressure at rest is significantly lower on the 5% level with ATP during exercise no significant difference is observed. The investigation shows that in many patients, especially younger ones, treatment with ATP makes it possible to obtain a larger Q during exercise, and thus to increase the oxygen transporting capacity of the circulation. This beneficial effect may be more pronounced in patients with low compliance of the ventricular myocardium.

In two earlier studies from this laboratory the effect of different ventricular rates on cardiac output and central pressure at rest and during exercise in patients with artificial pacemakers giving impulses at a fixed rate to the right ventricle has been reported (1, 3).

Atrial triggered ventricular pacing is now a well established technique for treatment of patients with AV block. There are however rather few studies of the haemodynamic effect of atrial triggered (ATP) versus fixed rate pacing (FRP) and most of them deal only with patients studied at rest (6, 18, 19). Center *et al.* (9) determined the cardiac output (Q) at ventricular FRP before implanting an ATP and with ATP 3-12 months after the operation. They demonstrated an increase in Q during atrial synchronous pacing both at rest and during exercise. Sowton (22) showed by comparing the haemodynamic effect of ventricular FRP to that of sinus rhythm that the connection of the pacemaker induced an immediate drop in Q following the loss of atrial delivery. In these studies however increases in stroke volume (SV) returned the Q to its original level during the next few minutes. In a preliminary study from this department the effect of atrial synchronization during work has been reported in two patients (15). This study indicated a beneficial haemodynamic effect of atrial synchronous pacing.

The present investigation deals with the haemodynamic effect of ATP versus ventricular FRP in a larger material. In one series 12 patients were investigated by cardiac catheterization, at rest and during work, using both FRP at about 70 beats/min and ATP. The same procedure was followed with both types of pacemakers. The main object was

Table 1 Some anthropometric data

Case no.	Sex	Age (y)	Height (cm)	Weight (kg)	BSA (m ²)	LV (ml)	Tt/b (g)
<i>Series I</i>							
1	♂	65	176	74	1.91	960	489
2	♂	65	170	68	1.80	1 100	544
3	♂	60	177	79	1.97	600	664
4	♂	66	175	91	2.06		761
5	♀	50	157	69	1.68	680	520
6	♂	56	175	64	1.80	1 160	493
7	♀	31	167	56	1.63	880	461
8	♀	67	151	64	1.58	490*	353
9	♂	51	170	73	1.84	1 320	625
10	♂	60	183	70	1.95	1 070	766
11	♀	49	163	76	1.81	900	569
12	♂	48	184	79	2.04	1 130	685
<i>Series II</i>							
1	♂	67	174	65	1.78	910	543
2	♀	66	164	76	1.82	880*	
3	♂	61	178	83	2.00	1 180	678
4	♀	30	158	55	1.55	755	349
5	♀	60	156	63	1.61	730*	366
6	♂	54	174	70	1.85	1 070*	
7	♂	64	161	75	1.78	1 300*	
8	♂	53	173	69	1.84	780*	
9	♀	45	161	63	1.65	770	362
10	♀	37	175	77	1.92	890	831
11	♂	70	182	87	2.10	1 080*	868
12	♂	65	179	86	2.06	860*	
13	♂	40	172	99	1.70	1 050	583

Examination made with patient in standing position.

study the circulatory dynamics during spontaneously increasing heart rate with synchronized atrial systole versus a fixed ventricular rate during work. In a second series of 13 patients the FRP was matched to the same rate as that previously obtained under identical conditions with an ATP in the same patient at rest and during work. This study demonstrates the effect of the atrial systole on the central haemodynamics.

MATERIAL

Series I

Twelve patients (4 women, 8 men), aged 31–67 years (mean 56) with complete AV block were studied. Each patient had a moderately enlarged heart (Table 1).

The duration of symptoms before electrode implantation and the duration of pacemaker treatment before the study as well as other relevant diseases are presented in Table II. It is evident that the duration of pacemaker treatment before the study is rather short in most patients, but in no case shorter than 15 days.

A history of rheumatic affection is suspected in three patients (nos. 3, 6, 12) of whom two have aortic stenosis. In

one patient (no. 2) the first spell of AV block III was recorded when he underwent treatment for a myocardial infarction. No definite aetiology could be established in the other seven patients.

Series II

Thirteen patients (5 women, 8 men), aged 30–70 years (mean 55) with complete AV block were studied. Each patient had an enlarged heart, but more markedly enlarged in only one (no. 7) who also had an aortic valvular lesion (Table I).

Table II shows that the duration of pacemaker treatment before the investigation is rather short in all patients, but shorter than III days in only one patient (no. 4). It appears from Table II that the aetiology of the AV block could be myocarditis in six patients (nos. 1, 3, 5, 6, 7, 9). Two of them (nos. 6, 7) have an aortic valvular lesion and one (no. 9) also has cardiomyopathy. Familial cardiomyopathy is present in three patients (nos. 4, 9, 13). In patients 2, 8, 10, 11 and 12 no definite aetiology could be established although one (no. 10) has hypertension and had a pulmonary tuberculosis at the age of 24.

The patients in series I and II are rather similar regarding age distribution and duration of pacemaker treatment. The mean duration of AV block before initiation of pacemaker treatment is longer in series I than in series II, a fact that should not interfere with the results. The patients in both

Table II Duration of symptoms before electrode insertion and duration of pacemaker treatment before the study

Case no.	Duration of symptoms before insertion of pacemaker electrode	Duration of pacemaker treatment before catheterization	Other relevant diseases
Series I			
1	2 mo.	1 mo.	
2	5 mo.	8 mo.	Infection 1 y ago short period ill AV III
3	5 y	1 mo	Rheumatic fever in 1930
4	6 y	15 d	
5	4 y	15 d.	
6	6 y	6 mo.	Aortic stenosis. In 1957 myocarditis
7	1 y	1 mo.	Air embolism 5 y ago
8	1 mo.	15 d.	Hypertonia
9	2 mo	2 mo.	Cardiac enlargement since 1 y
10	3 mo.	15 d.	
11	4 y	1 mo.	
12	10 y	1 y	Aortic stenosis
Series II			
1	5 mo.	15 d	Myocarditis 70 y ago
2	6 mo.	3 mo.	
3	6 mo.	15 d	Before onset of symptoms pleurisy and pneumonia
4	4 d.	6 d.	Hypertrophic cardiomyopathy
5	1 mo	15 d.	Diphtheria as child. Symptoms started after air-way infect. Myocarditis?
6	6 y	15 d	Rheumatic fever at the age of 35. Slight aortic incompet.
7	1.5 y	1 mo	Combined aortic lesion
8	20 d.	2 mo.	
9	2 y	1 mo.	In 1930 and 1936 rheumatic fever Cardiomyopathy
10	1 y	5 mo.	Pulmonary fib at the age of 24 Hypertonia
11	15 d	4 mo.	
12	4 mo.	1 mo.	
13	6 mo	3 mo	Cardiomyopathy

series belong to selected group suitable for ATP. Atrial fibrillation had never been observed in any of them. Some of the patients, when connected to the FRP temporarily showed interference of sinus rhythm. None had history of coronary insufficiency.

After being fully informed all patients consented to participate in the investigations.

METHODS AND PROCEDURE

In all patients transvenous monopolar electrode (Siemens-Elema, EMT 583) (14) had previously been introduced through the right external jugular vein, with its tip positioned in the apical region of the right ventricle. The electrode cable had been passed subcutaneously to the right groin, where it was cut out through the skin together with the cable of the indifferent electrode placed in the fatty tissue of the abdominal wall (16). In all patients except one (no. 3 series II) a P wave-sensing electrode had been implanted by means of mediastinoscopy (8) with its tip close to the atrial wall and its cable also drawn subcutaneously to the right groin, where it passed out through the skin. In the above mentioned patient P waves of an amplitude sufficient to trigger an atrial synchronous pacemaker were obtained with an oesophageal electrode during the study.

The studies of series I include recordings of central pressures and Q at rest and during work, with the patients in recumbent position connected to pacemaker giving a fixed rate of around 70 impulses/min (Siemens-Elema, EM 138). After resting for more than 30 min the patients were connected to an ATP (Siemens-Elema, EM 141) and the same procedure was repeated.

The patients in series II were investigated in the same way at rest and during work. In this series however the patients were first connected to the ATP and in the second half of the investigation the FRP was matched to the same impulse frequency as the ATP had given during the determination of Q both at rest and during work.

The choice of work load in both series was governed by the patients' performance during one or two work tests performed during the days before catheterization.

The same procedure was followed at every catheterization. In local anaesthesia double lumen catheter was introduced through cubital vein to the right heart and pulmonary artery and an arterial catheter was introduced percutaneously through the brachial artery and positioned with its tip in the ascending aorta. The reference level for zero pressure was at the mid-thoracic level. In no instance was the pacing electrode dislocated by the catheterization procedure. The pulmonary arterial wedge (PCV) pressure

was used as a measure of left atrial pressure. In patients in whom no wedge pressure was obtained the diastolic pulmonary artery (PA) pressure was used since this has been found to be almost the same in patients with normal pulmonary vascular resistance (PVR) (12).

Q was determined according to the Fick principle. The methods used in this laboratory for determination of heart volume (HV), blood gases, and for pressure measurement have previously been described (2). The determination of HV was performed with the patients connected to a pacemaker giving impulses at a fixed rate of 70/min. In most cases the examination was made with the patients in the supine position, irrespective of heart and respiratory cycle without angulation of the roentgen tube according to Kjellberg et al. (13) (Table I).

Total haemoglobin (THb) was determined by the alveolar CO method of Sjöstrand (21) modified by Holmgren (11). Normal values per kg b wt. are 10.23 (S.D. ± 1.18) for men and 8.07 (S.D. ± 0.85) for women.

RESULTS

In series I all patients were studied both at rest and during exercise. Patients 2 and 6 who could work without discomfort during FRP got angina pectoris during exercise on the same load during ATP and in no 2 the discomfort was so pronounced that the exercise test was stopped before Q could be determined.

In series II patients 2 and 4 were studied only at rest, all the others both at rest and during work. No patient felt any discomfort during the investigation.

Series I FRP versus ATP (Table III)

Heart rate (HR)

During ATP the HR at rest averaged 83 beats/min (S.D. ± 11.4). During FRP interference occurred at rest in three patients and the net HR averaged 72 beats/min (S.D. ± 4.5). During exercise and ATP at a work load averaging 383 kmp/min (S.D. ± 125) which increased the oxygen uptake (VO_2) to an average of 1034 ml/min (S.D. ± 247.8) the HR increased to an average of 126 beats/min (S.D. ± 11.5). During exercise and FRP interference rhythm occurred in five patients and the net HR was 83 beats/min (S.D. ± 15.4). VO_2 during the two experimental situations was almost identical.

Stroke volume (SV)

During ATP the SV at rest averaged 69 ml (S.D. ± 15.1) which is on an average 65% of the normal value predicted from the THb and 54% of

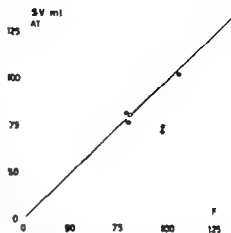


Fig. 1. Stroke volume (SV) at rest (○) and during exercise (●) and ATP in relation to SV at rest and during exercise at identical loads but with FRP in patients in series I.

the normal value predicted from the roentgenological HV in supine position. During exercise there was a significant increase in SV to 81 ml (S.D. ± 15.5) which is on an average 78% and 66% of the normal value predicted from the THb and HV respectively (Fig. 1).

During FRP the average SV at rest was 72 ml (S.D. ± 10.3) i.e. not significantly different from the SV obtained at rest during ATP. During exercise there was a marked increase in SV to an average of 104 ml (S.D. ± 14.8) which corresponds to the normal value predicted from the THb (107 ml) and 82% of the value predicted from the HV. The SV during exercise is highly significantly larger than the SV under identical conditions and ATP.

Cardiac output (Q)

During ATP Q at rest averaged 5.7 l/min (S.D. ± 0.9) and increased during exercise to an average of 9.8 l/min (S.D. ± 1.6). During FRP the Q at rest was on an average 5.2 l/min (S.D. ± 0.6) which is almost significantly lower than the Q at rest during ATP. During exercise and FRP Q increased to 8.1 l/min (S.D. ± 0.9) which is significantly less than during exercise and ATP (Fig. 2).

The relationship between Q and VO_2 at rest and during exercise is illustrated in Fig. 3. During ATP Q is within normal range comparable to that of normal subjects of almost the same age (10). During FRP Q is lower in relation to VO_2 than during ATP i.e. the same amount of oxygen is transported with a smaller Q .

Table III Results in series I (means \pm S.D.)

	HR (beats/min)	VO ₂ (ml STPD/min)	AVD (ml/l)	Q (l/min)	SV (ml)	Pressures (mmHg)			RV Systolic	End-diastolic	PVR index	SVR index
						Mean PCV or diastol. PA systol.	Aortic PA systol.					
<i>Rest</i>												
FRP	72 (± 4.5)	238 (± 40.2)	51 (± 7.4)	5.2 (± 0.6)	72 (± 10.3)	12.5 (± 3.5)	132 (± 19.2)	26.8 (± 4.4)	7.4 (± 2.1)	2.3 (± 0.99)	2.3 (± 0.99)	34.3 (± 6.18)
ATP	83 (± 11.4)	267 (± 38.7)	49 (± 7.2)	5.7 (± 0.9)	69 (± 15.1)	9.2 (± 4.5)	140 (± 20.5)	27.7 (± 8.5)	5.6 (± 2.4)	2.8 (± 1.45)	2.8 (± 1.45)	34.3 (± 6.49)
Difference	11	9	3	-0.5	3	3.3	-8	1.1	1.8	-0.5	-0.5	0.0
p	12	12	12	12	11	12	12	10	7	11	11	12
	<0.01**	>0.1**	<0.02*	<0.02*	>0.4**	<0.01	>0.1**	<0.6**	<0.05	>0.3**	>0.3**	>0.9**
<i>Work</i>												
FRP	83 (± 15.4)	1042 (± 251)	137 (± 24.1)	8.2 (± 0.9)	104 (± 14.8)	24.3 (± 6.2)	174 (± 17.3)	57.0 (± 11.8)	16.3 (± 4.3)	2.6 (± 1.37)	2.6 (± 1.37)	25.6 (± 2.17)
ATP	128 (± 11.5)	1034 (± 247)	107 (± 25.7)	9.8 (± 1.6)	81 (± 15.5)	23.9 (± 10.1)	169 (± 26.9)	54.4 (± 14.0)	12.7 (± 5.3)	2.7 (± 1.37)	2.7 (± 1.37)	23.6 (± 4.17)
Difference	45	90	30	-1.6	23	0.4	5	2.6	3.6	-0.1	-0.1	2.0
p	9	9	9	9	10	9	8	9	6	9	9	9
	<0.001	>0.7**	<0.01	<0.01**	<0.001	>0.6**	>0.3**	>0.2**	<0.05	>0.05**	>0.05**	>0.05**

** = non-significant, * = $p < 0.05$, ** = $p < 0.001$ Table IV Results in series II (means \pm S.D.)

	HR (beats/min)	VO ₂ (ml STPD/min)	AVD (ml/l)	Q (l/min)	SV (ml)	Pressures (mmHg)			RV Systolic	End-diastolic	PVR index	SVR index
						Mean PCV or diastol. PA systol.	Aortic PA systol.					
<i>Rest</i>												
FRP	80 (± 9.4)	263 (± 34.3)	60 (± 9.8)	4.5 (± 1.0)	57 (± 14.9)	14.1 (± 4.8)	136 (± 23.7)	30.3 (± 6.5)	8.5 (± 3.5)	2.8 (± 0.99)	2.8 (± 0.99)	43.1 (± 9.4)
ATP	77 (± 7.2)	282 (± 28.6)	51 (± 9.7)	5.3 (± 1.3)	69 (± 16.2)	12.2 (± 4.3)	147 (± 26.8)	31.9 (± 10.0)	7.6 (± 3.5)	2.6 (± 1.16)	2.6 (± 1.16)	40.3 (± 9.6)
Difference	3	19	9	-0.8	12	1.9	-11	1.6	0.9	0.2	0.2	2.8
p	12	12	12	12	1	9	11	7	8	8	10	10
	>0.05**	>0.9**	<0.001	<0.01	<0.01	<0.08	<0.05	>0.4**	>0.5**	>0.2**	>0.01	<0.05
<i>Work</i>												
FRP	121 (± 17.6)	1113 (± 267.8)	130 (± 17.1)	8.5 (± 1.6)	72 (± 16.9)	28.7 (± 5.9)	173 (± 18.9)	60.0 (± 12.3)	14.7 (± 7.0)	3.0 (± 1.05)	3.0 (± 1.05)	30.2 (± 8.6)
ATP	122 (± 19.5)	1135 (± 291.9)	124 (± 22.5)	9.2 (± 1.8)	77 (± 21.8)	28.1 (± 7.0)	174 (± 28.9)	61.7 (± 9.6)	18.4 (± 6.1)	3.3 (± 1.63)	3.3 (± 1.63)	27.6 (± 7.4)
Difference	1	-22	6	0.7	5	0.6	1	1.7	3.7	-0.3	-0.3	2.6
p	11	11	11	11	10	10	9	6	6	9	10	10
	>0.3**	>0.3**	>0.05**	<0.05	>0.1**	>0.7**	>0.1**	>0.5**	>0.1**	>0.7**	>0.7**	<0.05

Statistical symbol as in Table III

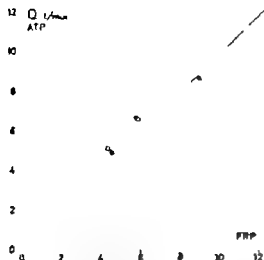


Fig. 2 Cardiac output (\dot{Q}) at rest and during exercise and ATP in relation to \dot{Q} at rest and during exercise at identical loads with FRP in patients in series I. Symbols as in Fig. 1.

Arteriovenous oxygen difference (AVD)

During ATP at rest the AVD averaged 48 ml/l (S.D. ± 7.2) which is higher than in normal young subjects but within the normal range for elderly patients (10). During exercise and ATP AVD increased more than in healthy elderly men. The AVD during FRP at rest averaged 51 ml/l (S.D. ± 7.4).

which is significantly higher than during ATP at rest (0.02). During exercise and FRP AVD increased significantly more with VO_2 than during exercise and ATP.

Filling pressure of the left ventricle (LV)

During ATP at rest the LV filling pressure, as estimated from mean pulmonary wedge (PCV) pressure or diastolic pulmonary arterial (PA) pressure averaged 9 mmHg (S.D. ± 4.5). During exercise it increased markedly to an average of 23.9 mmHg (S.D. ± 10.1).

During FRP at rest the filling pressure averaged 1.5 mmHg (S.D. ± 3.4) which is significantly higher than during ATP at rest. In both situations however this pressure is normal. During exercise the wedge pressure increased to 24.3 mmHg (S.D. ± 6.2) which is not different from the pressure level during ATP and exercise. This increase during exercise observed in both experimental conditions is within the normal range for healthy elderly males reported by Gramah *et al.* (10).

Systemic arterial pressure

The systolic aortic pressure or systolic LV pressure (in patient 12 who had an aortic stenosis) averaged 140 mmHg (S.D. ± 20.5) at rest and during ATP. Significant hypertension was not present in any patient. The increase during exercise was normal. During FRP no significant differences occurred compared to ATP. There was no significant difference in aortic mean pressure between FRP and ATP either at rest or during work.

Systemic vascular resistance (SVR)

The SVR index calculated according to the formula

$$\frac{\text{Paortic} - \text{BSA}}{\dot{Q}} \frac{\text{mmHg} \cdot \text{m}^2}{\text{l/min}}$$

(BSA = body surface area) was within normal range both at rest and during work. There was no significant difference in SVR between FRP and ATP.

Filling pressure of the right ventricle (RV)

During ATP at rest the end-diastolic pressure of the RV averaged 4.6 mmHg (S.D. ± 2.4) which is within the normal range (10). During exercise it increased to an average of 12.7 mmHg (S.D. ± 5.3) which still remains within the normal limits.

During FRP the RV end-diastolic pressure at rest averaged 7.4 mmHg (S.D. ± 2.1) which is slightly

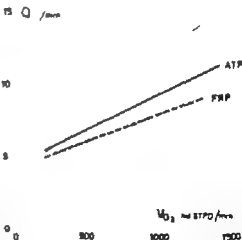


Fig. 3 Cardiac output (\dot{Q}) in relation to oxygen uptake (VO_2) at rest and during exercise in patients in series I and ATP (heavy full line) and FRP (heavy broken line). Regression lines for \dot{Q} on VO_2 in young healthy men (dotted line) and for elderly men (thin line) with the lower 95% confidence interval (thin broken line).

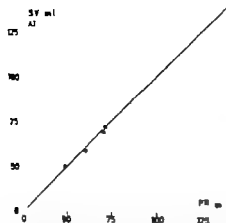


Fig. 4 Stroke volume (SV) at rest and during exercise and ATP in relation to SV at rest and during exercise at identical work loads but FRP at the matched frequency (FRPm) in patients in series II. Symbols as in Fig. 1

but significantly higher ($p < 0.05$) than during ATP. During exercise the end-diastolic pressure rises more and is slightly but significantly higher ($p < 0.05$) than during ATP.

Right ventricular and pulmonary arterial pressure

During ATP at rest systolic RV or systolic PA pressure averaged 28 mmHg (S.D. ± 8.5) which is within the normal range. During exercise there was a marked increase in the RV systolic pressure (54 mmHg, S.D. ± 14.0), which corresponds to the increase in the left LV filling pressure.

During FRP the systolic RV pressure was not significantly different either at rest or during work.

Pulmonary vascular resistance (PVR)

The PVR index calculated according to the formula

$$\frac{PPA - P_{PCV}}{Q} \quad \text{BSA} \frac{\text{mmHg} \cdot \text{m}^3}{\text{l/min}}$$

was at the upper normal limit both at rest and during work. There was no significant difference in PVR between FRP and ATP.

Series II: ATP versus frequency-matched FRP (FRPm) (Table IV)

Heart rate

During ATP the HR at rest averaged 77 beats/min (S.D. ± 7.2). During FRPm interference rhythm occurred in three patients and the resulting HR at rest averaged 80 beats/min (S.D. ± 9.0). During exercise and ATP at an average work load of 409 kmp/min

(S.D. ± 157.8) increasing the VO_2 to an average of 1135 ml/min (S.D. ± 291.9) the HR increased to 122 beats/min (S.D. ± 19.5). During exercise with FRPm interference rhythm occurred in three patients and the resulting HR averaged 121 beats/min (S.D. ± 17.6). VO_2 was almost identical with FRPm and ATP during exercise. With FRPm the VO_2 averaged 1113 ml/min (S.D. ± 267.8) during exercise.

Stroke volume

During ATP the SV averaged 69 ml (S.D. ± 16.2) which is on an average 64% and 54% of the normal value predicted from THb and HV respectively. During exercise there was a significant increase in SV to 77 ml (S.D. ± 21.8) which is on an average 71% and 60% of the normal value predicted from THb and HV respectively.

During FRPm the average SV at rest was 57 ml (S.D. ± 14.9), which is significantly less than during ATP. During exercise and FRPm SV increased significantly to 72 ml (S.D. ± 16.9) which is less but not significantly less than during ATP (Fig. 4).

Cardiac output

During ATP the \dot{Q} at rest averaged 5.3 l/min (S.D. ± 1.3) and increased during exercise to an average of 9.2 l/min (S.D. ± 1.8). During FRPm \dot{Q} at rest averaged 4.5 l/min (S.D. ± 1.0) which is highly significantly lower than at rest and with ATP. During exercise and FRPm \dot{Q} increased to 8.5 l/min (S.D. ± 1.6) which is less than with ATP and ever

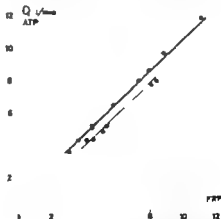


Fig. 5 Cardiac output (\dot{Q}) at rest and during exercise and ATP in relation to \dot{Q} at rest and during exercise at identical work loads but FRPm in patients in series II. Heavy line indicates least squares regression for all observations. Other symbols as in Fig. 1

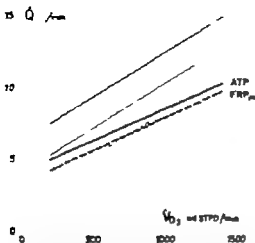


Fig. 6. Cardiac output (Q) in relation to oxygen uptake ($\dot{V}O_2$) at rest and during exercise in patients in series II and ATP (heavy full line) and FRPm (heavy broken line). Other symbols as in Fig. 3.

case on the 5% level (Fig. 5). The relationship between Q and $\dot{V}O_2$ under these circumstances is illustrated in Fig. 6, from which it is seen that the ATP patients tend to be less hypokinetic.

Arterio-venous oxygen difference

The AVD at rest during ATP averaged 51 ml/l (S.D. ± 9.7), which is higher than in normal young patients but almost within the normal range for 7 subjects (10).

During exercise and ATP the AVD increased as in patients in series I somewhat more than in elderly men (10) and averaged 1.4 ml/l (S.D. ± 22.5). The AVD during FRPm at rest averaged 60 ml/l (S.D. ± 9.8) which is significantly higher than during ATP. During exercise and FRPm AVD increased somewhat more but not significantly with $\dot{V}O_2$ than during ATP.

Filling pressure of the left ventricle

During ATP at rest the LV filling pressure as estimated from mean PCV pressure or diastolic PA pressure averaged 11 mmHg (S.D. ± 4.3). During exercise it increased markedly to an average of 28.1 mmHg (S.D. ± 7.0).

During FRPm at rest the filling pressure averaged 14.1 mmHg (S.D. ± 4.8), which is almost significantly higher than during ATP at rest. In both situations however the PCV pressure is close to normal. During exercise the wedge pressure increased to 28.7 mmHg (S.D. ± 5.9) which is not different from the pressure level at ATP. This increase during exer-

cise observed in both experimental conditions is only a little higher than in healthy elderly men as reported by Granath et al. (10).

Systemic arterial pressure

The systolic aortic pressure averaged 147 mmHg (S.D. ± 26.8) at rest and during ATP. The increase during exercise was normal. During FRPm at rest the pressure was somewhat lower than during ATP whereas during work there was no significant pressure difference between the two experimental conditions. Significant hypertension was present in two patients (nos. 5 and 10). There was no significant difference in aortic mean pressure between FRPm and ATP either at rest or during work.

Systemic vascular resistance

The average SVR index was high at rest, but decreased to the upper normal level during work. Both at rest and during work the SVR was slightly significantly lower with ATP than with FRPm.

Filling pressure of the right ventricle

During ATP at rest the end-diastolic pressure of the RV averaged 7.6 mmHg (S.D. ± 3.5) which is within the normal range (10). During exercise it increased to an average of 18.2 mmHg (S.D. ± 6.1), which is above the normal range.

During FRPm the RV end-diastolic pressure averaged 8.5 mmHg (S.D. ± 3.5), which is not significantly higher than during ATP. During exercise the end-diastolic pressure rises to the same level as with ATP.

Right ventricular and pulmonary arterial pressure

During ATP at rest the systolic RV or systolic PA pressure averaged 32 mmHg (S.D. ± 10.0) which is only slightly above the normal range of variation. During exercise there was a marked increase in the RV systolic pressure to 62 mmHg (S.D. ± 9.6), which corresponds to the increase in the LV filling pressure.

During FRPm the systolic RV pressure was not significantly different either at rest or during work.

Pulmonary vascular resistance

The average PVR index was at the upper normal range both at rest and during work. There was no significant difference in PVR between FRPm and ATP.

DISCUSSION

In the present study atrial triggered pacing has been compared with fixed rate pacing by determination of the central haemodynamics at rest and during work, in patients with AV block selected for treatment with ATP. This implies that all patients had a normal sinus rhythm of the atria. Atrial fibrillation had never been recorded in any patient. Some of the patients had AV block III only during shorter or longer periods. During the study three patients in series I showed interference from conducted sinus rhythm at rest and five during work, when connected to the FRP.

In series II three patients showed interference from conducted sinus rhythm both at rest and during work, when the FRP was connected. This interference between sinus rhythm and pacemaker induced fixed rate certainly influences the results in making the difference in haemodynamic effect of atrial triggered versus fixed rate pacemaking less pronounced.

Another factor influencing the results of the studies is the state of the myocardium. Most patients treated with pacemakers are elderly but for this study rather young patients were selected. The mean age was 56 in series I and 51 in series II. As indicated in Table II many patients had other cardiac lesions with possible myocardial dysfunction and this might also be the case in some of the other patients. This implies that both elastic and contractile properties of the atrial and ventricular myocardium could have been impaired in many of the patients.

This study of the haemodynamic effect of ATP versus FRP is divided into two parts. Series I is a study in which the effect of ATP is compared to the effect of stimulation with FRP at a rate of about 70 impulses/min. Hence the stimulation with ATP implies not only synchronicity between atrial and ventricular contraction, but also an increase in ventricular rate during work. Series II on the other hand is an attempt to study the pure effect of the synchronized atrial contraction on the pump function of the ventricles. The effect of rate in this series is eliminated by adjusting the frequency of the FRP both at rest and during work, to almost the same as that obtained when the ATP was connected. With this planning of the investigation in series II it follows that the study with ATP in every patient had to precede the study with fixed or asynchronous pacemaker. This may introduce a systematic error which, however, is judged to be of minor importance

as the patients were allowed to rest for half an hour after the first work test before the next study was performed.

ATP versus FRP at rest

The effect of synchronous versus asynchronous pacing on cardiac output at rest was studied under almost the same conditions in both series.

In series I the average ventricular rate with FRP was 72 and with ATP 83 beats/min whereas in series II it was somewhat higher with FRPm (80 beats/min) than with ATP (77 beats/min).

On an average the Q with ATP is 10% higher in series I and 18% higher in series II as compared to FRP. The significance of the difference is low in series I ($p < 0.02$) somewhat higher in series II ($p < 0.01$). The difference in increase in Q with ATP between the two series could be explained by interindividual variations in a small material. The small differences in HR between FRP and ATP cannot be the explanation, as this should result in a larger difference in series I than in series II. Therefore the increase in Q with ATP (10–18%) at rest must be explained by synchronization of atrial systole. This increase in Q is in agreement with the results of other authors. Samet et al. (19) demonstrated in six patients that Q was about 10% higher during synchronous pacing at rest compared with ventricular pacing, although the interindividual variations were pronounced also in their material.

Wiseheart et al. (23) studied the effect of ventricular and atrial pacing at rest, over a wide range of pacing rates in 13 patients with stable sinus rhythm after open heart surgery. They did not find any difference in Q with atrial pacing at different rates compared to sinus rhythm. With ventricular pacing Q was 17% lower than with atrial pacing. As remarked by these authors, the reason for this difference is not only the asynchronicity of atrial contraction with ventricular pacing. With atrial pacing the excitation of the ventricles occurs via normal pathways resulting in a normally coordinated ventricular contraction, while excitation initiated at the implanted ventricular electrode results in an uncoordinated ventricular contraction. In the present study the ventricles were paced with the same ventricular electrode both at ATP and fixed rate pacing thus eliminating the effect of different activation patterns.

The interindividual variations in the effect of ATP on Q were large. An example of this is patient

- 5 Brockman, S. K. Dynamic function of atrial contraction in regulation of cardiac performance. *Amer J Physiol.* 204: 597 1963
- 6 Brockman S K., Collins, H. A., Bloomfield D. A., Sinclair-Smith, B. C. & Gobbel W. B. Physiological studies and clinical experience in patients with synchronous and asynchronous pacemakers. *J thorac. cardiovasc. Surg.* 51: 864 1965
- 7 Barcheff, H. B. A clinical appraisal of atrial transport function. *Lancet* 1: 775 1964
- 8 Carless E.. Mediastinoscopy. A new method for inspection and tissue biopsy in the superior mediastinum. *Dis. Chest* 36: 343 1959
- 9 Center S., Nathan, D., Wu C. Y. & Dugue D. Two years of clinical experience with the synchronous pacer. *J thorac. cardiovasc. Surg.* 48: 513 1964
- 10 Granath A., Jonsson, B. & Strandell, T. Circulation in healthy old men studied by right heart catheterization at rest and during exercise in supine and sitting position. *Acta med. scand.* 176: 425 1964
- 11 Holmgren, A.. Total hemoglobin, techniques normal values relation to fitness, values in heart patients. *Mal. cardiovasc.* 10: 1 1967
- 12 Joernson B. & Sanael S. The reliability of diastolic pressure measurement in the pulmonary artery as an index of mean left atrial pressure. *Cardiologia* 34: 329 1969
- 13 Kjellberg, S. R., Löneroth H. & Rudbe U. The effect of various factors on the roentgenological determination of the cardiac volume. *Acta radiol.* 35: 413 1951
- 14 Lagergren H. & Johansson, L., Intracardiac stimulation for complete heart block. *Acta chir. scand.* 125: 562, 1963
- 15 Lagergren H. & Johansson L., Karlöf I. & Thorsander H. Atrial-triggered pacemaking without thoracotomy: Apparatus and results in twenty cases. *Acta chir. scand.* 132: 678 1966.
- 16 Lagergren H. & Karlöf I. Pacemaking according to a system of building blocks. *J. thorac. cardiovasc. Surg.* 56: 51 1968.
- 17 Larsson, S., Alestig, K., Bojs, O. & Bergh N. P. Treatment by atrial-triggered pacemaker. *Scand. J. thorac. cardiovasc. Surg.* 3: 186, 1969
- 18 Martin R. H. & Cobb L. A. Observations on the effect of atrial systole in man. *J. Lab. clin. Med.* 68: 224 1966
- 19 Samet, P., Bernstein W., Nathan D. D. & Lopez, A.: Atrial contribution to cardiac output in complete heart block. *Amer J. Cardiol.* 16: 1 1965
- 20 Saranoff S. J., Gilmore, J. P. & Mitchell J. H. Influence of atrial contraction and relaxation on closure of mitral valve: Observations on effects of autonomic nerve activity. *Circulation Res.* 11: 26, 1962.
- 21 Sjöstrand T. A method for determination of total hemoglobin content of the body. *Acta physiol. scand.* 16: 211 1948
- 22 Sowton, E. Artificial pacemaking and sinus rhythm. *Brit. Heart J.* 27: 311 1965
- 23 Wisheart, J. D., Wright, J. E. C., Rosenfeldt, F. L. & Ross, J. K. Atrial and ventricular pacing after open heart surgery. *Thorax* 28: 9 1973

INITIAL SERUM POTASSIUM LEVEL IN RELATION TO EARLY COMPLICATIONS AND PROGNOSIS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

T Dyckner C. Helmers T Lundman and P O Wester

From the Medical Department, Karolinska Institute at Serafimerlasarettet
Stockholm Sweden

Abstract. During two years 450 patients with AMI have been treated in the CCU at Serafimerlasarettet. Serum potassium level was determined in 444 patients on admission. Hyperkalaemia was a rare finding associated with bad state on admission and a poor prognosis. Hypokalaemia was recorded in 15% of the patients and was associated with previous diuretic treatment, supraventricular bradycardia as well as atrial flutter and fibrillation during the first 24 hours in the CCU. Ventricular ectopic beats and ventricular tachycardia were also seen more frequently in hypokalaemic than in other patients.

Cardiac function and arrhythmias in relation to changes in serum potassium concentration have been extensively investigated in experimental and clinical studies. Investigations of the incidence of early complications and arrhythmias in relation to initial serum potassium in patients with acute myocardial infarction (AMI) are scarce. As treatment with saluretics of patients in age groups with a high incidence of AMI is common, a hypokalaemic tendency in some of these patients may be expected on admission to hospital. An evaluation of early possible and prognostic implications of such findings has been the aim of this investigation.

PATIENTS AND METHODS

In 1968 and 1969 450 patients with AMI, 264 men (59%) mean age 63 years, and 166 women (37%), mean age 71 years were treated in the Coronary Care Unit (CCU) at Serafimerlasarettet, Stockholm. The CCU mortality was 10% and the total hospital mortality 21%. A detailed description of the CCU as well as the criteria for admission and diagnosis have been presented elsewhere (3). Serum potassium was determined on admission in 444 cases. A conventional technique with autoanalyser was used. The normal values were 3.6-5.1 mEq/l. For testing the signifi-

cance of differences of proportions the χ^2 -test was used, almost significant 0.01 < p < 0.05, significant 0.001 < p < 0.01.

Definitions

Left heart failure (LHF). Basal rales, a third heart sound or X-ray findings of central vascular enlargement. **Frank pulmonary oedema.** Patients with rales heard all over the chest in association with frothy sputum. **Hypotension.** A systolic BP of 90 mmHg or below. **Shock.** Hypotension in combination with clinical signs of shock such as cold skin, deterioration of sensation, oliguria. **Supraventricular tachycardia (SVT).** Sinus tachycardia and nodal tachycardia as well as atrial tachycardia, flutter and fibrillation with QRS rate \geq 100 beats/min. **Supraventricular bradycardia (SVB).** Sinus bradycardia, nodal rhythm or atrial flutter and fibrillation (AF) with QRS rate < 50 beats/min. **First degree heart block (A-V I).** Sinus rhythm with PQ intervals > 0.22 sec. **Ventricular tachycardia (VT).** A rhythm with 3 or more ventricular ectopic beats (VEB) in succession.

RESULTS

The patients were divided into four groups according to their initial serum potassium levels. 64 patients (15%) had < 3.6 mEq/l, 263 (59%) 3.6-4.3 mEq/l, 103 (23%) 4.4-5.1 mEq/l and 14 patients (3%) had a potassium value > 5.1 mEq/l. Only 4 patients had values < 3.0 mEq/l and none > 6.0 mEq/l.

Hypokalaemia was significantly more common in patients above than below 70 years of age. It was also a more common finding in women than in men. As could be anticipated, previous medication with diuretics was also significantly more frequent in hypokalaemic (43%) than in normokalaemic patients (27%). Corresponding figures for previous digitalis medication were 35 and 31% respectively.

Normokalaemic patients had a total hospital mor-

- analysis of clinical state and blood gas changes. *Amer Heart J* 79: 620 1970.
2. Fisch C., Knochel S., Feigenbaum, H. & Greenspan, K.. Potassium and the monophasic action potential electrocardiogram, conduction and arrhythmias. *Progr cardiovasc Dis.* 8: 387 1966.
3. Helmers, C. Short and long-term prognostic indices in acute myocardial infarction. *Acta med. scand Suppl.* 555 1973.
4. Helmers, C., Hofvendahl S., Lundman, T., Mogensen L., Nyquist, O., Silve U. & Wester P. O. Arterial oxygen and carbon-dioxide tension in patients with acute myocardial infarction. *Cardiology* 58: 335 1973.
5. Helmers, C., Mogensen, L., Nordlander R., Orinäs, E., Sjögren, A. & Wester P. O. Acid-base disturbances in patients with acute myocardial infarction. *Acta med. scand.* 194: 241 1973.
6. Huth, E. J. & Squines, R. D. The relation of cardiovascular phenomena to metabolic change in a patient with chronic hypokalemia. *Circulation* 14: 60 1956.
7. Kirby B. J. & McNicol M. W. Acid-base states in acute myocardial infarction. *Lancet* 2: 1054 1966.
8. Ljungström B., Johansson, B. W. & Sievers, J. Arterial pO_2 , pCO_2 , pH and standard bicarbonate in patients with an acute myocardial infarction. *Cardiology* 51: 138 1967.
9. Surawicz, B. Electrolytes and the electrocardiogram. *Mod. Conc. cardiovasc Dis.* 38: 875 1964.
10. — Role of electrolytes in etiology and management of cardiac arrhythmias. *Progr cardiovasc Dis.* 8: 364 1966.
11. Surawicz, B. & Lepeschkin, E. The electrocardiographic pattern of hypopotassemia with and without hypokalemia. *Circulation* 8: 801 1953.

INCIDENCE AND PRESENTATION OF MYOCARDIAL INFARCTION IN NORTH KARELIA FINLAND

Pekka Puska and Harri Mustaniemi

*From the Coordinating Centre of North Karelia Project University of Kuopio,
Kuopio Finland*

Abstract A myocardial infarction (MI) register was started on May 1 1972 in the county of North Karelia in Eastern Finland as part of the North Karelia project. Information about all cases with suspected acute MI among the North Karelian population are collected by the register. The principles of the register follow the recommendations of the WHO expert working group. Altogether 713 cases were registered between May 1 and Dec. 31 1972. The distribution of them into diagnostic categories was: definite 57% possible 30% "no acute MI" 8% and "insufficient information" 5%. Patients with no acute MI have been excluded in the results. About 47% of the male patients below 65 years had history of previous MI. Most of the patients in the group had been heavy cigarette smokers, eating mainly butter as the fat in their diet. Overweight was rare among the male patients but not among the females. During the years preceding the attack, most of the patients had visited physician and pathological ECG had been recorded. The average time delay before hospital treatment was internationally relatively short. The 4-week fatality rate among patients below 65 years was 37% for males and 35% for females. These rates were slightly lower than those in the register material in Helsinki. The annual incidence rate per thousand for the age group 30-64 in North Karelia was 13.8 among males and 2.6 among females. The incidence rate increased continuously with age among males, among females it increased markedly only after the age of 60. The risk ratio between North Karelia and Helsinki for the age standardized incidence rates of males in the age group 30-64 was 1.38 and for respective mortality rates 1.21. Within North Karelia the highest incidence rate for males aged 30-64 was recorded in the rural area of Ilomantsi-Tuusula in the East.

The high morbidity and mortality rates of coronary heart disease (CHD) in Finland in general and particularly among the middle-aged men living in the county of North Karelia, Eastern Finland, have been known since the 1950s (7). Since then several

statistical calculations and the so-called "Seven Countries Study" have confirmed these observations (1 5 6 8 9).

A growing concern among the North Karelian population about the high frequency of CHD resulted in 1971 in a petition from the representatives of the population to the national government to take measures to reduce the burden of the disease. After the planning stage and with the WHO's assistance a comprehensive preventive programme of cardiovascular disease (CVD) on a community basis was established. The intermediate objectives of this North Karelia project are a) to reduce the general level of the known risk factors of CVD among the population and b) to promote early diagnosis, treatment and rehabilitation of patients with CVD. The background, aims and methods of the project have been described elsewhere (10).

From the very beginning of the planning of the project it was felt necessary to establish a register to collect uniform information about all possible cases of acute myocardial infarction (MI) and stroke in the county of North Karelia (180 000 inhabitants). These registers started accordingly together with the intervention on May 1 1972. The experience gained by the WHO and some of the collaborating centres (2, 4 11-16) could be used for the methodology of the MI register.

The aim of the MI register of the North Karelia project is to serve the preventive programme by collecting information on 1) the occurrence of the disease, the natural history of the disease and the features typical of the patients and 2) the function of the health care system at the occurrence of the disease attacks. The information in the register is used both for continuous planning of the primary as well as secondary preventive programmes and for

Part of these results have been presented in Puska: "North Karelia project—a programme for community control of cardiovascular diseases" (acad. diss.) Kuopio 1974.

Table 1 Division of the material into diagnostic categories

	Males				Females			
	<64 y		≥65 y		<64 y		≥65 y	
	N	%	N	%	N	%	N	%
Definite	208	60.5	93	60.0	33	47.1	73	50.7
Possible	99	28.8	48	31.0	25	35.7	41	28.5
No acute MI	26	7.6	8	5.2	7	10.0	13	9.0
Insufficient data	11	3.2	6	3.9	5	7.1	17	11.8
Total	344	100.0	155	100.0	70	100.0	144	100.0

assessing the results. The register serves the evaluation of the project by measuring both the incidence of the disease and some of the intermediate objectives of the programme.

THE REGISTER

Information about all cases suspected of MI among the North Karelian population is collected by the register. The principles of the register follow the recommendations of the WHO expert working group (16). As soon as possible after notification of a case an *initial record form* is completed. At discharge from hospital or if still in hospital, 4 weeks after the onset of symptoms, a *follow-up record form* is completed. For fatal cases *death record form* is also completed. Half a year as well as one year after the onset of symptoms postal enquiry is made about the patient's condition.

The register centre is situated at the Central Hospital of North Karelia which treats about 2/3 of all acute MI patients in the county. A full-time nurse completes the record of all MI patients treated at the Central Hospital. The remaining MI patients are treated at small hospitals mainly attached to local health centres. At each local hospital one or two appointed nurses complete the record forms of all their MI patients. The local hospitals send their record forms immediately to the register centre together with more detailed information on the diagnostic criteria (pain, enzymes and ECG) and the diagnosis established by the treating physician. At the register centre the interest of the register (H.M.) checks all the record forms and confirms the diagnostic conclusions.

The nurse of the register centre keeps in close contact with the local register nurses and checks periodically all death certificates of the county. She receives information from the local health insurance office and the hospital dismissal cards of the patients treated for CHD. Health workers of the county are continuously informed about the principles and results of the register through the general information on the project to these workers.

According to the principles of the WHO expert group and the collaborating centres, the cases are divided into the diagnostic categories "definite", "possible", "no acute MI" or "insufficient data". The last category consists of some dead patients in whom acute MI as the cause of death

can be neither confirmed nor ruled out on the basis of available information. The diagnostic conclusions are based on information about pain history, enzyme tests, ECG and autopsy (14, 16).

MATERIAL

The material consists of the patients registered during the first year of the register i.e. in whom symptoms started between May 1 and Dec. 31 1972. Of the 713 cases registered during this period 57% fell into the diagnostic category definite, 30% into "possible", 8% into "no acute MI" and 5% into "insufficient data" (Table 1). The category "no acute MI" has always been excluded in the final analysis; the category "insufficient data" has been included only when results concerning the incidence, mortality and fatality rates are presented. The material includes all cases of acute MI: first infarctions and reinfarctions, in the community during the investigation period. Twenty-three persons had had an attack twice and two persons three times during the period.

For the categories "definite" and "possible" a pain history was available in 81% and an ECG in 74% enzyme test results in 70% and an autopsy diagnosis in 11% of the cases which is 27% of the dead patients. The missing information was usually due to sudden or early death. Of this available information the results were considered to be typical of acute MI for pain in 86% for ECG in 38% for enzymes in 76% and for autopsy in 94% of the cases.

In about 2/3 of the cases the first source of notification was the arrival in hospital. In the event of such an attack, most patients in North Karelia come directly to the hospital. About 1/3 of the patients were dead before registration. Eighty per cent of the patients came from rural areas. The male patients below 65 years were mainly farmers or lumbermen (39%), industrial workers (25%), or pensioners (21%).

RESULTS

Previous history and risk factors

Among the males 47% of those below 65 years and 50% of those above 65 had had a previous MI, most of them only one. For the females the attack was

Table II Fatality rates during the periods from the onset of symptoms to the first medical examination and the 28th day

Period	Fatality rate (%)			
	Males		Females	
	<64 y	≥65 y	<64	≥65 y
From the onset to				
The first medical examination	26	12	25	28
The 28th day	37	60	35	49
Total	318	147	63	131

more frequently their first. About 70% of the younger males had suffered from previous angina of effort, 20% from diagnosed hypertension and 7% from diabetes. These data were based on the available information given by the patients and not always confirmed by the records or by a physician.

A positive family history of MI or stroke was more common among younger than older patients. Among the younger male patients it was more common on the father's than on the mother's side (45%/25%). For the female patients the ratio was reverse (31%/43%).

At the time of the attack 65% of the younger male patients were current smokers, a percentage which is clearly higher than that for the general male population of the same age in North Karelia (11), 11% of them had never smoked regularly. The smokers smoked on an average about 20 cigarettes a day. Smoking was much less frequent among the younger female patients (20%). Among the younger male patients 70% had used mainly butter as the fat in their diet. Overweight, expressed by a "body mass" index of at least 31, was present in only 8% of the younger male patients compared with 35% of the younger female patients.

About 2/3 of the younger male patients had visited a physician at least six times during the previous three years. Only 6% of them had not visited a physician at all during that period. The blood pressure had been measured during the previous three years in 86% of the male and in 94% of the female patients. Younger females had more often than younger males been told that their BP was elevated (58%/22%). An ECG had been recorded in 83% of all patients. 68% of them had been informed of its abnormality.

Onset of symptoms and delay in medical care

Information about prodromal symptoms 28 days before the onset of the attack was collected by the register. In 68% of the male patients below 65 years the common prodromal symptom was fresh or exacerbation of previous angina of effort and more than half of these patients had felt discomfort in the chest and heaviness in the arm. Among the younger female patients the most common prodromal symptom was discomfort in the chest (85%). In the representative sample of the general North Karelian population at the baseline survey of the project about 2/3 of both males and females aged 45-59 stated that they had angina or discomfort of effort and the same proportion had experienced pain or discomfort in the chest during the previous year (11). About 70% of the attacks started at home. Less attacks (19%) were recorded to have started during the first six hours of day than during the other hours. No major differences were found between the frequency of attacks and the month of onset.

Information about the delay in receiving medical aid was collected in the record form. In these items a lot of information was however missing due to the large number of early deaths (23% of the younger male patients had died before the first medical examination) and in several cases it was difficult to determine the exact time of the onset of symptoms (either because of the gradual start or the nervousness of the family). Thus among the younger male group information was missing about "patient delay" in 53% and about "delay due to conditions" in 38%.

From the information obtained it was calculated that the median delay for the younger male patients in summoning medical aid ("patient delay") was less than two hours. The median delay from summoning medical aid to arrival in the hospital ("delay due to conditions") was for the same group slightly less than one hour. About half of the patients were transported by ambulance and the other half by taxi or private car.

The patient delay for younger male patients was less than two hours in 59% of all cases (on whom information was obtained). In 57% of the reinfarctions and in only 48% of the cases with worsening angina as a prodromal symptom. Thus previous infarction did not apparently shorten the delay in summoning medical aid. Angina of effort seemed even to lengthen the delay in some cases.

Table III Annual age and sex specific incidence rates per thousand inhabitants

Age (y)	Males		Females	
	N	Incidence rate	N	Incidence rate
30-34	3	0.9	1	0.3
35-39	5	1.4	1	0.3
40-44	22	5.8	5	1.4
45-49	61	17.8	6	1.6
50-54	59	19.9	11	3.1
55-59	72	24.6	16	4.6
60-64	96	33.4	23	6.8
65-69	67	33.7	48	18.4
70-74	48	37.2	43	23.2
75-79	21	32.3	20	18.4
80-	11	27.7	20	27.0
Total	465	14.9	194	5.8

Prognosis

Death before first medical examination was frequent in all groups (Table II). The 4-week fatality rate even if high in the younger male group, was hardly of the European average and below the 42% fatality rate in the Helsinki register (12, 15).

Nearly half (47%) of all younger male patients had been seen by a physician during the fortnight preceding death. Nearly all of them had seen a physician several times during the previous three years. An ECG had been recorded in 91% of the same younger fatal male group. 73% of the whole group had been told it was abnormal. Previous angina, angina of effort and smoking were more common in the fatal group than in the total material of males below 65 years.

A multiple discriminant analysis was performed in the group of male patients below 65 years of age with the diagnoses definite or 'possible acute MI' to find out whether it might be possible to differentiate the group of survivors from the group of fatal cases on the basis of the existing information on the situation before the attack. Eight variables were accepted in the analysis. When only those cases were included who provided information on all eight variables, the number of cases in the survivor group was reduced from 201 to 184 and in the fatal group from 106 to 66. This analysis showed that the total discriminatory power of the chosen variables was good ($F(8,241)=5.256$). The two variables that predicted the death best were previous ECG (pathological) and previous MI in the history (positive). Working in the same direction, although less markedly, were smok-

ing and use of butter. Age, overweight (body mass index) and previous angina of effort had practically no discriminatory power. In this material a history of hypertension was slightly more common in the non-fatal than in the fatal group.

Incidence and mortality

In the calculation of the incidence and mortality rates official population data from Dec. 31, 1971 were used. These data include corrections on the basis of the national census in 1970. The age- and sex-specific incidence and mortality rates are shown in Tables III and IV. The incidence rate among the males increased gradually with age, among the females there was a sharp increase only in old age.

The incidence and mortality rates have been compared with the results of the MI register in Helsinki (13). Because of differences in age structures of the two populations, age standardization was made using the North Karelian population as standard. The annual incidence rates per thousand for males aged 30-64 were 13.8 for North Karelia and 10.0 for Helsinki, the risk ratio being 1.38. The respective figures for mortality rates were 5.1 and 4.2, the ratio being 1.21.

The incidence rates in North Karelia for the different health centre areas were calculated. Although there is considerable random variation due to the rather small absolute numbers, the differences were marked. The urban capital of the county had the annual rate of 12.1 ($N=53$) and the rural area of Ilomantsi-Tuusula in the East had the rate of 21.8 ($N=42$) for males aged 30-64, calculated per

Table IV Annual age and sex specific mortality rates per thousand inhabitants

Age (y)	Males		Females	
	N	Mortality rate	N	Mortality rate
30-34	-	-	-	-
35-39	2	0.6	1	0.3
40-44	8	2.1	1	0.3
45-49	19	5.4	-	-
50-54	23	7.8	5	1.5
55-59	28	9.6	5	1.4
60-64	38	13.2	9	2.7
65-69	39	19.6	20	7.4
70-74	29	22.5	18	9.8
75-79	14	1.6	11	10.1
80-	6	15.1	15	20.3
Total	205	6.6	87	2.4

thousand inhabitants. The latter area is the Eastern Finnish area in the "Seven Countries Study" (6).

DISCUSSION

The MI register as part of the comprehensive community preventive programme of CVD serves the programme in several ways as described in the introduction. This article concerns the first year of the MI register of the North Karelia project. The emphasis is accordingly on the feasibility, coverage, comparability and description of the baseline situation.

The MI register in North Karelia covers—at least compared with the registers collaborating in the WHO study—a great geographical area (18 000 km²) with a low population density. This caused some worry about the feasibility of the register at the planning stage. The experience is, however, encouraging. Collection of information does not require very great resources. Integration with the official health services and the use of the information channels of the project are obviously of great help. So is the rather low mobility of the population. On the other hand much remains to be done to increase the quality and quantity of the information in many cases, to increase the number of autopsies and to improve the diagnostic remarks in medical records and death certificates. Also it is relatively laborious to build up a comprehensive information system where the information of the MI register is linked with other sources of information on a continuous and personal basis.

The coverage of the register is considered to be good. The well followed recommendation is that all patients suspected of an acute MI are treated in hospital. Cooperation between the register nurse and the responsible nurses at the local hospitals is continuous. A great majority of the cases are registered soon after the attack. In about 15% of the cases the death certificate is the source of information. Other sources of ensuring the coverage have so far yielded only a few cases.

For the comparability of the diagnostic conclusions inside the county all the registered cases are reviewed and final conclusions are made by the register physician. For the comparability with other centres the principles of the WHO working group are followed as carefully as possible (16). People responsible for the register have been in contact with the WHO and its collaborating centres especially the Finnish ones. The register nurse had training at

another Finnish centre prior to the start of the registration.

The register results seem to confirm the previous data on the high MI mortality and incidence rates in North Karelia. The age standardized incidence rate among the middle-aged males seems to be nearly 1.4-fold compared with that of Helsinki which had the clearly highest figure in the collaborating centres of the WHO study (16, 17). The picture does not change when the comparison is restricted to cases with definite diagnosis of acute MI: the definite cases calculated as a percentage of all cases are about the same in the two register materials (65% vs. 63%).

The finding is new that the 4-week fatality rate seems to be an exception in the generally unfavourable natural history of the disease in North Karelia. This might of course, be due to a registration of more numerous milder cases than e.g. in Helsinki on the other hand, because of the distances and the general assumption that the North Karelian men do not easily consult a physician, this might not be true. A lower fatality rate could be explained by the general high physical activity of North Karelian men who mainly have a physically heavy occupation (3).

Preliminary results seem to show that there are considerable differences in incidence rates in North Karelia, the rate being generally higher in the rural areas. This would be in agreement with the finding that the general high level of the CHD risk indicators is even higher in rural than in urban North Karelia (11).

The results have several implications in the preventive programme. The high level of the primary risk indicators, frequent heavy smoking and consumption of animal fat, fit in agreement with the objectives of the primary preventive programme (10). The findings of the history of morbidity, frequent medical contacts and diagnosed coronary symptoms lay emphasis on the intensification of the preventive work by the general practitioners at the local health centres. The high number of reinfarctions lays special emphasis on the secondary preventive programme.

Less benefit in the programme might be expected as to the treatment of the acute phase. About 2/3 of the cases and usually the younger as well as the complicated cases are treated at the Central Hospital with a coronary care unit. Simultaneously the median time delay in transferring the

hospital is remarkably short, especially considering the long distances. This, of course, does not mean that shortening of the time delay is not necessary and possible in many cases. The internationally relatively short average delay seems to be due to the prevailing system in which most emergency patients of this type are transferred directly to the hospital. The rather short patient delay might also be due to the high awareness of the problem among the population. On the other hand, due to the high morbidity the various prodromal symptoms of the patients are also very common among the general population of the county according to the surveys made by the project. This reduces the value of the prodromes in the preventive programme.

The registration in North Karelia continues. As the number of registered cases increases, more detailed epidemiological analyses can be made concerning the occurrence of MI in North Karelia. When these data are linked with those from the baseline survey of the project (representative sample of the North Karelia population) conclusions can be drawn about the risk indicators among this population. Simultaneously the register will show during the following years trends in the parameters that are subject to the intervention and thus give feedback to the programme.

REFERENCES

1. Bolander A.-M. A comparative study of mortality by cause in four Nordic countries, 1966-1968, with special reference to male excess mortality. *Statist. Rep. B* 1971. 9 Stockholm 1971.
2. Fodor J. The ischaemic heart disease register in Örebro. A pilot study 1.1.1968-31.1.1969. *Peik. Dubb.* 26, 1969.
3. Frank, C. W., Weibull, O., Shapiro, S. & Sager, R. V. Physical inactivity as a lethal factor in myocardial infarction among men. *Circulation* 34: 1022, 1966.

4. Parberg, G., Lundqvist, L. & Svärdsudd, K., Preventive health program in Norrbotten with special reference to heart diseases. *Acta sociomed. scand. Suppl.* 6: 200, 1972.
5. Hiltö A. S. Kuolleisuus Suomessa ja muissa Pohjoismaissa 1948-1964. *Duodecim* 82: 1136, 1966.
6. Keys, A. (ed.) Coronary heart disease in seven countries. *Circulation Suppl.* 16: 1, 1970.
7. Keys, A., Karvonen M. J. & Pkkanen, F. Serum cholesterol studies in Finland. *Lancet* 2: 175, 1958.
8. Leppo K., Lindgren, J. & Rintamäki, M. Mortality trends in Finland in the 1960's. *Yearbook of population research in Finland*. XII, 1971. Vammala 1972.
9. Puska, P. Sydän- ja verisuonitautien ehkäisyn tutkimusalueiden alueelliset erot. I. *Suom. Lääk.-L.* 27: 3071, 1972.
10. — The North Karelia project, an attempt at community prevention of cardiovascular diseases. *WHO Chronicle* 27: 55, 1973.
11. Puska P., Rimpelä, M., Siivola, K., Tuomilehto, J., Virtamo J., Parnila, T. & Kumpulainen, Y. Pohjois-Karjala projektin peruskartoitus: toteutus ja perustulokset. Kuopion korkeakoulun julkaisu, kansanterveysosasto II 1/1973.
12. Rosio M. Factors related to sudden death in acute ischaemic heart disease. A community study in Helsinki. Helsinki 1972.
13. Rosio M., Siitonen P., Mäkelä, V., Pyörälä, K. & Halonen, P. The mortality from coronary disease in Helsinki on the basis of WHO ischaemic heart disease register. *Scand. J. clin. Lab. Invest. Suppl.* 116: 18, 1971.
14. Ruostemäki, R. Sydäninfarktin diagnostiikka. *Suom. Lääk. L.* 27: 3197, 1972.
15. WHO. The role of mobile coronary care units. Report of a Working Group. WHO/EURO 3620 (2), Moscow 3-4.2.1970.
16. — Ischaemic heart disease registers. Report of the Fifth Working Group. WHO/EURO 3201 (5), Copenhagen 26-29.4.1971.
17. — The prevention and control of major cardiovascular diseases. Report of a Conference. WHO/EURO 3214 Brussels 1973.

SUBSTRATE INCORPORATION INTO HEPATIC LIPIDS AND PROTEINS IN VITRO IN PATIENTS WITH PRE- β HYPERLIPOPROTEINEMIA

H Stakeberg and T Schersten

*From the Departments of Medicine I and Surgery II Sahlgrenska sjukhuset
University of Göteborg Göteborg S. eden*

Abstract Fifty-three patients operated on for uncomplicated gallstone disease have been studied concerning the hepatic synthesis rate *in vitro* of triglycerides and proteins. Thirteen of the patients had pre- β hyperlipoproteinemia. Five of them and four normolipoproteinemic patients were fed sucrose-enriched diet for 14 weeks prior to the operation. In the non-sucrose-fed hyperlipoproteinemic patients the liver concentration of triglycerides (TG) and the incorporation rate of precursors into TG were increased. A significant correlation was found between the synthesis rate of TG in liver tissue and the plasma TG concentration in these hyperlipoproteinemic patients. After sucrose feeding of patients with hyperlipoproteinemia the concentration of phosphoglycerides (PG) the incorporation rate of labelled precursors into PG were significantly lower than in normolipoproteinemic patients and in hyperlipoproteinemic patients on an ordinary diet. The incorporation rate of leucine into hepatic proteins and the hepatic protein concentration were the same in non-sucrose-fed controls, sucrose-fed and non-sucrose-fed hyperlipoproteinemic patients. The results indicate an increased vulnerability of the hepatic PG and protein metabolism for dietary sucrose in patients with pre- β hyperlipoproteinemia.

The role of the liver in the development of the increased plasma concentration of very low density lipoproteins (VLDL) in patients with endogenous hyperlipoproteinemia has been debated (5, 8, 10, 11, 18, 21, 22). In a recent study we obtained some evidence for an involvement of the hepatic lipid metabolism in pre- β hyperlipoproteinemic patients (6). Sucrose feeding of these patients was associated with significantly lower capacity for hepatic phosphoglyceride (PG) synthesis. However, whether this changed hepatic lipid metabolism was

an inherent metabolic error in the liver of these patients specifically provoked by the sucrose diet could not be settled.

More recent findings of an altered hepatic lipid and protein metabolism in patients with extra-hepatic cholestasis provide further evidence for a contributive role of hepatic metabolism in dyslipoproteinemic conditions (25). In the cholestatic patient with increased concentration of low density lipoproteins (LDL) in serum, the synthesis rate of PG and proteins in liver tissue was increased.

The purpose of the present study was to further elucidate the role of the liver in pre- β hyperlipoproteinemia. Hyperlipoproteinemic patients were compared with normolipidemic patients with respect to the capacity *in vitro* of liver tissue for lipid and protein synthesis. Some of these patients were fed sucrose-enriched diet for 14 days before the investigation.

MATERIAL AND METHODS

Clinical material. The study group consisted of 13 patients, 7 men and 6 women aged 55 (± 3) years. The patients, who all had pre- β hyperlipoproteinemia (types II B and IV) according to Fredrickson and Lees (12) and Beaumont et al. (2), were admitted to the hospital for operation of uncomplicated gallbladder disease and in one patient for atherosclerotic abdominal aortic disease. Clinical data pertinent at the time of admission are given in Table 1. The plasma triglyceride (TG) and cholesterol concentrations were significantly higher than in randomly selected men, born in 1913 and living in the same city as the study group (4). Five of these patients (nos 9-13) were fed sucrose-enriched diet for 14 days before the operation according to our previous description (6).

Table I Clinical data on the study group at the time of admission

Pat. no	Age (y)	Sex	B wt. (kg)	Plasma TG (mmol/l)	Plasma cholesterol (mg/100 ml)	Lipoprotein type
			height (cm) - 100			
1	62	♂	1.16	3.4	771	IV
	50	♂	1.38	3.2	319	IV
3	58	♂	1.08	2.7	314	IV
4	72	♂	0.93	2.7	333	IIIB
5	61	♀	1.02	2.3	369	IIIB
6	50	♂	1.03	3.0	281	IV
7	60	♀	1.13	2.5	340	IIIB
8	61	♂	1.19	2.5	283	IV
9	24	♀	0.78	2.0	215	IV
10	60	♂	1.11	2.6	295	IV
11	51	♀	1.29	2.2	211	IV
12	51	♀	1.07	3.8	393	IIIB
13	57	♀	1.13	2.2	255	IV
Mean \pm S.E.			1.10 \pm 0.04	2.7 \pm 0.2	399 \pm 15	

As controls served 40 patients: 11 males aged 54 (\pm 3) years and 29 females aged 49 (\pm 3) years, all admitted to the hospital for an operation of uncomplicated gallbladder disease. Four of the controls (nos 14-17) were fed sucrose-enriched diet for 14 days before the operation.

All patients in the study group had normal liver function according to our previous definition (6). None of the control patients had any known disorder apart from the gallbladder disease. Patients with known or apparent alcohol problems and women taking oral contraceptive steroids were excluded from the study. The plasma TG (1.3 ± 0.1 mmol/l) and cholesterol (217 ± 12 mg/100 ml) concentrations in the control group did not differ from middle-aged men and women from the same city (3). No statistically significant difference was found in relative body weight between the study group (1.10 ± 0.05) and the controls (1.02 ± 0.04).

The plasma lipoprotein pattern was evaluated from repeated plasma lipid analyses. Lipoprotein electrophoresis on agarose gel (23) and from α -lipoprotein cholesterol determinations (2) as described previously (6).

Experimental procedures. All patients fasted from 8 p.m. the day before until the operation at approximately 8 a.m. They were premedicated one hour before introduction of general anaesthesia by means of diazepam and atropine. Anaesthesia was given as hexobarbital (Evipan®) nitrous oxide, oxygen and succinyl choline.

The liver biopsy was performed immediately after the abdominal cavity had been opened as described previously (20). The preparation and incubation of liver slices for determination of the incorporation rate of glycerol and fructose into liver lipids and glycogen was performed according to earlier descriptions (6, 20). For determination of the incorporation rate of leucine into proteins, liver

Table II Concentrations of triglycerides, phosphoglycerides, glycogen and proteins in liver slices from patients in the study and control group

Patient no.	TG (μ mol g ⁻¹)	PG (μ mol g ⁻¹)	Glycogen (mg g ⁻¹)	Proteins (mg g ⁻¹)
Pre β-hyperlipoproteinemia				
1	13.3	24.2	4.4	59.0
2	19.9	21.1	3.6	34.4
3	59.8	19.8	4.4	56.0
4	6	31.7	2.6	79.6
5				62.6
6	12.2	21.1	6.1	60.2
7	58.3	21.1	3.7	34.6
8	11.6	26.4	3.8	71.8
Mean \pm S.E.	25.9 \pm 8.7	23.6 \pm 1.6	4.7 \pm 0.5	62.2 \pm 3.2
Controls				
Mean \pm S.E.	10.1 \pm 2.8	23.3 \pm 0.9	3.8 \pm 0.6	57.8 \pm 1.4
n	14	15	8	20
P	<0.05	n.s.	n.s.	n.s.

Table III Concentrations of triglycerides, phosphoglycerides and glycogen in liver slices from patients in the study and control groups after sucrose feeding for 14 days

Pat. no.	TG ($\mu\text{mol g}^{-1}$)	PG ($\mu\text{mol g}^{-1}$)	Glycogen (mg g ⁻¹)
<i>Pre-β hyperlipoproteinemia</i>			
9	—	21.5	3.4
10	36.3	1.3	11.1
11	34.9	18.0	10.7
12	138.7	19.0	4.0
13	12.1	18.7	6.7
$\bar{x} \pm \text{S.D.}$	55.5 ± 28	19.7 ± 0.7	7.1 ± 1.6
<i>Controls</i>			
14	3.0	27.7	4.7
15	4.1	20.9	5.7
16	38.2	23.9	3.7
17	56.6	16.8	7.5
\bar{x}	25.5	22.3	5.4

slices were incubated for 4 hours in complete amino acid mixture (supplied by AB Astra Södertälje) at final concentration four times that of human serum as described in a previous paper (24).

Analytical procedures The lipid phosphorus determinations were performed according to Bartlett (1) as modified by Srennerholm and Vreder (26). Glyceride glycerol was determined as described by Carlson (7). Neutral lipids and phospholipids were separated according to Gloster and Fletcher (13). Glycogen was determined according to van

der Vies (27). Protein was assessed according to Lowry et al. (15). Determination of the radioactive incorporation into TG, PG, into the fatty acid (FA) moiety of glycerides and into glycogen was performed as described previously (20). Determination of the radioactive incorporation into protein was performed according to our previous description (24).

The counting of radioactivity was performed in a Packard-Tri-Carb (3320) liquid scintillation spectrometer. Correction for quenching was performed by the external standard method.

Statistical methods The Student's *t*-test was applied for the comparison of mean values of different groups. Linear regressions were calculated according to the method of least squares and standard procedures were used to calculate correlation coefficients. *T*-tests were applied to check the significance of the regression coefficients.

RESULTS

Hepatic content of lipids, glycogen and proteins In patients with pre- β hyperlipoproteinemia on an ordinary diet prior to operation the liver tissue may contain more TG than that of controls (Table II). The concentrations of PG, glycogen, and proteins did not differ from those of the controls.

The five sucrose-fed hyperlipoproteinemic patients (nos. 9–13) showed high TG concentration $55 \pm 28 \mu\text{mol/g}$, low PG concentration $19.7 \pm 0.7 \mu\text{mol/g}$, high glycogen concentration, $7.2 \pm 1.6 \text{ mg/100 mg}$, and normal protein concentration (Ta-

Table IV Incorporation rate of glycerol and fructose into triglyceride, phosphoglycerides, glyceride, fatty acids, glycogen and of leucine into proteins in liver slices from patients on an ordinary diet and controls

Pat. no.	Glycerol ($\mu\text{mol h}^{-1} \text{g}^{-1}$)				Fructose ($\mu\text{mol h}^{-1} \text{g}^{-1}$)				Leucine ($\mu\text{mol h}^{-1} \text{g}^{-1}$)
	TG	PG	FA	Glycogen	TG	PG	FA	Glycogen	Protein
<i>Pre-β hyperlipoproteinemia</i>									
1	329.6	105.6	8.6	11.7	108.2	34.2	19.7	7.2	0.26
2	363.5	91.0	2.7	8.6	83.6	30.2	4.0	17.1	0.13
3	351.8	118.6	5.9	17.7	76.2	42.3	12.3	77.1	0.35
4	197.5	110.0	2.6	18.0	39.3	79.3	5.3	22.0	0.20
5	—	—	—	—	—	—	—	—	0.40
6	199.4	78.0	5.8	10.0	77.9	30.4	14.4	7.5	0.39
7	237.0	80.1	2.7	17.8	67.1	23.6	6.2	4.4	0.40
8	228.5	83.5	1.2	21.4	15.2	28.6	2.7	22.9	0.33
Mean \pm S.E.	258.2 ± 23.1	95.3 ± 6.1	4.2 ± 1.0	15.0 ± 1.8	77.1 ± 6.2	31.2 ± 2.1	9.2 ± 2.4	18.3 ± 3.1	0.37 ± 0.05
<i>Controls</i>									
Mean \pm S.E.	209 ± 15	82.6 ± 6.4	2.6 ± 0.3	11.8 ± 0.9	89.9 ± 9.0	23.0 ± 3.0	5.3 ± 0.8	19.8 ± 3.1	0.39 ± 0.04
<i>p</i>	16	16	18	4	16	16	16	4	20
	<0.05	<0.10	<0.10		n.s.	<0.025	<0.10		n.

Table I Clinical data on the study group at the time of admission

Pat. no	Age (y)	Sex	B wt. (kg)	Plasma TG (mmol/l)	Plasma cholesterol (mg/100 ml)	Lipoprotein type
			height (cm) - 100			
1	62	♂	1.16	3.4	271	IV
2	50	♂	1.38	3.2	319	IV
3	58	♂	1.08	2.7	314	IV
4	72	♂	0.93	2.7	335	IIIB
5	61	♀	1.02	2.3	369	IIIB
6	50	♂	1.03	3.0	281	IV
7	60	♀	1.13	2.5	340	IIIB
8	61	♂	1.19	2.5	283	IV
9	4	♀	0.78	2.0	215	IV
10	60	♂	1.11	2.6	295	IV
11	51	♀	1.29	2.2	211	IV
12	51	♀	1.07	3.8	393	IIIB
13	57	♀	1.13	2.2	255	IV
Mean \pm S.E.			1.10 \pm 0.04	2.7 \pm 0.2	299 \pm 15	

As controls served 40 patients, 11 males aged 54 (± 3) years and 29 females aged 49 (± 3) years, all admitted to the hospital for an operation of uncomplicated gallbladder disease. Four of the controls (nos 14-17) were fed sucrose-enriched diet for 14 days before the operation.

All patients in the study group had normal liver function according to our previous definition (6). None of the control patients had any known disorder apart from the gallbladder disease. Patients with known or parent alcohol problems and women taking oral contraceptive steroids were excluded from the study. The plasma TG (1.3 ± 0.1 mmol/l) and cholesterol (217 ± 12 mg/100 ml) concentrations in the control group did not differ from middle-aged men and women from the same city (3). No statistically significant difference was found in relative γ weight between the study group (1.10 ± 0.05) and the controls (1.02 ± 0.04).

The plasma lipoprotein pattern was evaluated from repeated plasma lipid analyses, lipoprotein electrophoresis on agarose gel (23) and from α -lipoprotein cholesterol determinations (2) as described previously (6).

Experimental procedures. All patients (fasted from 8 p.m. the day before until the operation at approximately 8 a.m.) were premedicated one hour before introduction of general anaesthesia by means of diazepam and atropine. Anaesthesia was given as hexobarbital (Evipan®), nitrous oxide, oxygen and succinyl choline.

The liver biopsy was performed immediately after the abdominal cavity had been opened as described previously (20). The preparation and incubation of liver slices for determination of the incorporation rate of glycerol and fructose into liver lipids and glycogen were performed according to earlier descriptions (6, 20). For determination of the incorporation rate of leucine into proteins, liver

Table II Concentrations of triglycerides, phosphoglycerides, glycogen and proteins in liver slices from patients in the study and control group

Patient no	TG (μ mol g ⁻¹)	PG (μ mol g ⁻¹)	Glycogen (mg g ⁻¹)	Proteins (mg g ⁻¹)
<i>Pre β-hyperlipoproteinaemia</i>				
1	13.3	24.2	4.4	59.0
2	19.9	21.1	5.6	54.4
3	59.8	19.8	4.4	56.0
4	62	31.7	2.6	79.6
5	-	-	-	62.6
6	12.2	21.1	6.1	60.2
7	58.5	21.1	5.7	54.6
8	11.6	26.4	3.8	71.8
Mean \pm S.E.	25.9 \pm 8.7	23.6 \pm 1.6	4.7 \pm 0.3	62.2 \pm 3.2
<i>Controls</i>				
Mean \pm S.E.	10.1 \pm 2.8	23.3 \pm 0.9	3.8 \pm 0.6	57.8 \pm 1.4
P	11	15	8	20
P	<0.05	n.s.	n.s.	n.s.

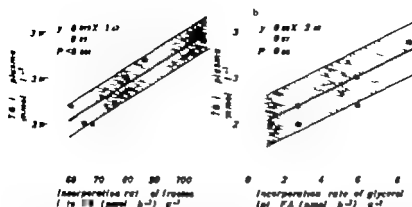


Fig. 1 Correlations between the incorporation rate of fructose into liver triglycerides and plasma triglyceride concentration (a) and between the incorporation rate of glycerol into the fatty acid moiety of glycerides (b) in liver

tissue and the plasma triglyceride concentration in pre- β hyperlipoproteinemic patients on an ordinary diet (b). The hatched area indicates \pm S.D.

above the upper 95% confidence limit of the non-sucrose-fed controls.

Correlations In the pre- β hyperlipoproteinemic patients on an ordinary diet bivariate regression analyses showed a correlation between the incorporation rate of fructose into hepatic TG and the plasma TG concentration (Fig. 1a). There was also a correlation between the incorporation rate of glycerol into the FA moiety of glycerides and the plasma TG concentration (Fig. 1b). The corresponding correlation for the fructose incorporation into the FA moiety of glycerides showed a trend to significance ($r=0.61$, $p<0.10$). No correlation was found between the incorporation rate of precursors into hepatic TG and the TG concentration in the plasma or the relative body weight in the controls.

DISCUSSION

The hepatic production and secretion rates of VLDL in hyperlipoproteinemic patients have been estimated by several methods (5, 8, 10, 11, 14, 17, 18, 21, 22). Some of these studies have indicated that the liver is of major importance for the hypertriglyceridemia in these patients (10, 11, 18, 21) but others have arrived at an opposite conclusion (5, 8, 14, 22). These contradictory results can probably be ascribed to more or less pronounced methodological errors as recently pointed out by Nikkilä and Kekki (18). The present approach to the problem was an effort to determine directly the hepatic capacity of patients with pre- β

hyperlipoproteinemia to synthesize TG, PG, FA and proteins. This *in vitro* method is for obvious reasons also affected with limitations, particularly concerning the interpretation of the results in a quantitative sense. However, the conditions for the determinations are identical for the study group and the controls, so that qualitative differences should be adequately disclosed.

The present studies showed only slight differences of the hepatic lipid and protein metabolism in patients with pre- β hyperlipoproteinemia as compared with normal controls. More pronounced differences were found when pre- β hyperlipoproteinemic patients on an ordinary diet were compared with those fed a sucrose-enriched diet prior to the liver tissue sampling. In the former the TG concentration in liver tissue was increased. This increase would suggest an enhanced hepatic synthesis of TG. The finding of a higher incorporation rate of glycerol into TG in these patients supports this hypothesis as does the correlation between incorporation rate into TG and plasma TG concentration. These findings agree with those of Olefsky et al. (21) and Nikkilä and Kekki (19) of a higher TG turnover (measured by endogenous 3 H-glycerol labelling technique) in patients with endogenous hypertriglyceridemia. Based on their observations Nikkilä and Kekki (19) suggested that the primary defect in most cases of type IV endogenous hypertriglyceridemia is an increased inflow transport of plasma TG. However, in our recent study on the effects of a sucrose-rich diet on

the hepatic lipid synthesis and the plasma removal capacity (6) we obtained evidence for both an increased hepatic production of TG and a decreased removal rate from plasma.

The most intriguing finding in our previous study (6) on the effects of sucrose feeding in pre- β hyperlipoproteinemia was a lower hepatic PG content and a reduced capacity for hepatic PG synthesis. This finding was confirmed in a small group of patients in this study. Moreover the present studies showed that in the hyperlipoproteinemic patients on an ordinary diet the capacity for PG synthesis was enhanced. The interpretation of these findings may be that the hepatic PG metabolism in patients with pre- β hyperlipoproteinemia (often referred to as carbohydrate-induced hyperlipemia) is specifically vulnerable to sucrose-rich diet. To what extent this metabolic error in the liver contributes to the dyslipoproteinemia in these patients cannot be decided from the present results.

In patients with hyperlipoproteinemia, the hepatic protein synthesis as evaluated by the incorporation rate of leucine into soluble liver proteins, did not differ from that of controls. However in the control patients who were fed a sucrose-rich diet prior to the investigation the incorporation rate of leucine was high as compared with non-sucrose-fed controls. However this small case series does not permit any unambiguous conclusion concerning the effect of sucrose feeding on the hepatic synthesis of proteins. In the rat it has been shown that the lipoprotein synthesis is enhanced by long-term glucose feeding (9). A stimulation of protein synthesis, an increased level of plasma insulin may explain the effect of carbohydrate diet (16). The lack of response of the hepatic protein synthesis rate to sucrose-rich diet in the patients with hyperlipoproteinemia may be another aspect of increased vulnerability of the hepatic metabolism for dietary sucrose in patients with pre- β hyperlipoproteinemia.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish National Association against Heart and Chest Diseases, the Swedish Medical Research Council (project no. 536), the Swedish Cancer Society (project no. 93) and from the Medical Faculty, University of Göteborg.

REFERENCES

- 1 Bartlett, G. R. Phosphorus assay in column chromatography. *J. biol. Chem.* 234: 466, 1959.

- 2 Beaumont, J. L., Carlson, L. A., Cooper, G. R., Fejar, Z., Fredrickson, D. S. & Strasser, T. Classification of hyperlipoproteinemias and hyperlipidemias. *Bull. Wild Hlth Org.* 43: 891, 1970.
- 3 Björntorp, P., Bengtsson, C., Blohme, G., Johansson, A., Sjöström, L., Tibblin, E., Tibblin, B. & Wåhlin, L. Adipose tissue fat cell size and number in relation to metabolism in randomly selected middle-aged men and women. *Metabolism* 20: 977, 1971.
- 4 Björntorp, P., Berchtold, P. & Tibblin, G. Insulin secretion in relation to adipose tissue in man. *Diabetes* 20: 63, 1971.
- 5 Bohberg, J. Mechanisms of hypertriglyceridemia in man. Studies of the metabolism of blood plasma triglycerides. A methodological and clinical investigation. *Acta Un. upsalen.* 105, 1971.
- 6 Cahlin, E., Jönsson, J., Persson, B., Ståleberg, H., Björntorp, P., Gustafsson, A. & Schersten, T. Sucrose feeding in man. Effects on substrate incorporation into hepatic triglycerides and phosphoglycerides in vitro and on removal of intravenous fat in patients with hyperlipoproteinemia. *Scand. J. clin. Lab. Invest.* 32: 21, 1973.
- 7 Carlson, L. A. Determination of serum triglycerides. *Acta Soc. Med. upsalen.* 64: 208, 1959.
- 8 Exton, R. Ph. Synthesis of plasma triglycerides in endogenous hypertriglyceridemia. *J. Lipid Res.* 12: 491, 1971.
- 9 Exton, R. Ph. & Alpmis, H. Effect of glucose feeding on lipoprotein synthesis in the rat. *Amer. J. Physiol.* 217: 1153, 1969.
- 10 Farquhar, J. W., Goss, R. C., Wagner, R. M. & Reaven, G. M. Validation of an incompletely coupled two-compartment nonrecycling catenary model for turnover of liver and plasma triglycerides in man. *J. Lipid Res.* 6: 119, 1965.
- 11 Fine, M., Michaels, G., Shah, S., Choi, B., Felekyan, H. & Kimmell, L. The incorporation of C¹⁴ from uniformly labeled glucose into plasma triglycerides in normals and hypertriglyceridemics. *Metabolism* 11: 893, 1966.
- 12 Fredrickson, D. S. & Lees, R. S. A system for phenotyping hyperlipoproteinemia. *Circulation* 31: 321, 1975.
- 13 Ghossein, J. & Fletcher, R. F. Quantitative analysis of serum lipids by thin-layer chromatography. *Clin. chem. Acta* 11: 235, 1966.
- 14 Havel, R. J., Kane, J. P., Balasie, E. O., Segel, N. & Basso, L. V. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoprotein in normotriglyceridemic and hypertriglyceridemic humans. *J. clin. Invest.* 49: 2017, 1970.
- 15 Lowry, O. H., Rosebrough, N. J., Farr, L. A. & Randall, R. J. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* 193: 265, 1951.
- 16 Manchester, K. L. Effect of insulin on protein synthesis. *Diabetes* 21: 447, 1972.
- 17 Nikkila, E. A. Control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin. *Advanc. Lipid Res.* 7: 63, 1969.

- 18 Nikkila, E. A. & Kakki, M. Measurement of plasma triglyceride turnover in the study of hyperglyceridaemia. *Scand. J. clin. Lab. Invest.* 27 97 1971.
- 19 — Plasma endogenous triglyceride transport in hypertriglyceridaemia and effect of hypolipidaemic drug. (SU-13437). *Europ. J. clin. Invest.* 2: 31 1972.
- 20 Nilsson, S. & Schersten, T. Synthesis of phospholipids and triglycerides in human liver slices I. Experimental conditions and the synthesis rate in normal liver tissue. *Scand. J. clin. Lab. Invest.* 24 237 1969.
- 21 Olefsky, J., Ferguson, J. W. & Reaven, G. H. Sex difference in the kinetics of triglyceride metabolism in normal and hypertriglyceridaemic human subjects. *Europ. J. clin. Invest.* 4, 121 1974.
- 22 Qvarfordt, S. H., Frank, A., Stenman, E. M., Berman, M. & Steinberg, D. Very low density lipoprotein triglyceride transport in type IV hyperlipoproteinaemia and the effects of carbohydrate-rich diets. *J. clin. Invest.* 49: 2281 1970.
- 23 Rapp, W. & Kahlös, R. Lipoprotein-electrophoresis in agarosegel. *Chim. chim. Acta* 19: 493 1968.
- 24 Stakeberg, H., Gustafson, A. & Schersten, T. Incorporation rate of leucine into proteins in human liver slices. *Europ. J. clin. Invest.* 4 393 1974.
- 25 Stakeberg, H., Lundborg, H. & Schersten, T. Incorporation rate in vitro of precursors into hepatic lipids and proteins in patients with extrahepatic cholestasis. *Europ. J. clin. Invest.* 4 399 1974.
- 26 Svennerholm, L. & Vanders, M. T. The distribution of lipids in the human nervous system. II Lipid composition of human fetal and adult brain. *Brain Res.* 47 457 1972.
- 27 van der Vies, J. J. Methods for determination of glycogen in liver. *Biochem. J.* 57 410, 1954.

the hepatic lipid synthesis and the plasma removal capacity (6) we obtained evidence for both an increased hepatic production of TG and a decreased removal rate from plasma.

The most intriguing finding in our previous study (6) on the effects of sucrose feeding in pre- β hyperlipoproteinemia was a lower hepatic PG content and a reduced capacity for hepatic PG synthesis. This finding was confirmed in a small group of patients in this study. Moreover, the present studies showed that in the hyperlipoproteinemic patients on an ordinary diet the capacity for PG synthesis was enhanced. The interpretation of these findings may be that the hepatic PG metabolism in patients with pre- β hyperlipoproteinemia (often referred to as carbohydrate-induced hyperlipemia) is specifically vulnerable to sucrose-rich diet. To what extent this metabolic error in the liver contributes to the dyslipoproteinemia in these patients cannot be decided from the present results.

In patients with hyperlipoproteinemia, the hepatic protein synthesis, as evaluated by the incorporation rate of leucine into soluble liver proteins, did not differ from that of controls. However, in the control patients who were fed a sucrose-rich diet prior to the investigation, the incorporation rate of leucine was high as compared with non-sucrose fed controls. However, this small case series does not permit any unambiguous conclusion concerning the effect of sucrose feeding on the hepatic synthesis of proteins. In the rat it has been shown that the lipoprotein synthesis is enhanced by long-term glucose feeding (9). A stimulation of protein synthesis and an increased level of plasma insulin may explain

an effect of carbohydrate diet (16). The lack of response of the hepatic protein synthesis rate to sucrose-rich diet in the patients with hyperlipoproteinemia may be another aspect of increased vulnerability of the hepatic metabolism for dietary sucrose in patients with pre- β hyperlipoproteinemia.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish National Association against Heart and Chest Diseases, the Swedish Medical Research Council (project no. 536), the Swedish Cancer Society (project no. 98) and from the Medical Faculty, University of Göteborg.

REFERENCES

1. Barden, G. R. Phosphorus assay in column chromatography. *J. biol. Chem.* 234: 466, 1959.

2. Beaumont, J. L., Carlson, L. A., Cooper, R., Fekkar, Z., Fredrickson, D. S. & Strasser, T. Classification of hyperlipoproteinemias and hyperlipidemias. *Bull. Wild. Hlth. Org.* 43: 891, 1970.
3. Björntorp, P., Bengtsson, C., Björnsen, G., Johansson, A., Sjöström, L., Tibblin, B., Tibblin, G. & Wilhelmsen, L. Adipose tissue fat cell size and number in relation to metabolism in randomly selected middle aged men and women. *Metabolism* 20: 977, 1971.
4. Björntorp, P., Berthold, P. & Tibblin, G. Insulin secretion in relation to adipose tissue in man. *Diabetes* 20: 65, 1971.
5. Bobberg, J. Mechanisms of hypertriglyceridemia in man. Studies of the metabolism of blood plasma triglycerides. A methodological and clinical investigation. *Acta Un. scandinav.* 105, 1971.
6. Cahlin, E., Jönsson, J., Persson, B., Stakeberg, H., Björntorp, P., Gustafsson, A. & Schersten, T. Sucrose feeding in man. Effects on substrate incorporation into hepatic triglycerides and phospholipids in vitro and on removal of intravenous fat in patients with hyperlipoproteinemia. *Scand. J. clin. Lab. Invest.* 32: 21, 1973.
7. Carlson, L. A. Determination of serum triglycerides. *Acta Soc. Med. scandinav.* 208: 1959.
8. Easton, H. Ph. Synthesis of plasma triglycerides in endogenous hypertriglyceridemia. *J. Lipid Res.* 12: 491, 1971.
9. Easton, R. Ph. & Kipalis, D. H. Effect of glucose feeding on lipoprotein synthesis in the rat. *Amer. J. Physiol.* 217: 1153, 1969.
10. Farquhar, J. W., Gross, R. C., Wagner, R. M. & Reaven, G. H. Validation of an incompletely coupled two-compartment recirculating cannula model for turnover of liver and plasma triglycerides in man. *J. Lipid Res.* 6: 119, 1965.
11. Fine, M., Michaels, G., Shah, S., Chaf, B., Finkels, M. G. & Kinzell, L. The incorporation of C^{14} from uniformly labeled glucose into plasma triglycerides in normals and hypertriglyceridemia. *Metabolism* 11: 893, 1966.
12. Fredrickson, D. S. & Lees, R. S. A system for phenotyping hyperlipoproteinemia. *Circulation* 31: 321, 1975.
13. Ghossein, J. & Fletcher, R. F. Quantitative analysis of serum lipids with thin-layer chromatography. *Clin. chim. Acta* 11: 235, 1966.
14. Havel, R. J., Kane, J. H., Balese, E. O., Segel, N. & Basile, L. V. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J. clin. Invest.* 49: 2017, 1970.
15. Lowry, O. H., Rosebrough, N. J., Farr, L. A. & Randall, R. J. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* 193: 265, 1951.
16. Manchester, K. L. Effect of insulin on protein synthesis. *Diabetes* 11: 447, 1972.
17. Nikkila, E. A. Control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin. *Advanc. Lipid Res.* 7: 63, 1969.

ANTIBODY TITRE CHANGES AND SKIN REACTIVITY IN PATIENTS WITH LIVER CIRRHOSIS UNDERGOING PORTOCAVAL SHUNT OPERATION

Kirsten Stehr Johansen, Torben Stehr Johansen and Jørgen Leerhey

From Statens Serum Institut Smallpox Vaccine Department Copenhagen and Department of Surgical Gastroenterology S Gentofte Hospital, H Herup Denmark

Abstract. Ten patients with liver cirrhosis undergoing portocaval shunt operation have been followed immunologically during their postoperative course regarding antibody titres to various antigens: viral as well as bacterial. The antibody determinations included rubella, vaccinia and cytomegalo viruses, diphtheria toxoid, *Candida albicans*, streptolysin O, typhoid and paratyphoid O and H and the syphilis reactions: Kahn, Wassermann and Meinelike. Twenty-one blood donors served as controls. Skin test reactions to diphtheria, *Candida albicans*, streptokinase and tuberculin were performed on the same patients. Eight patients submitted to cholecystectomy served as controls for pre and postoperative skin tests and antibody titres. The liver cirrhosis group before operation had significantly higher number of elevated antibody titres concomitant with significantly reduced skin test reactivity to diphtheria toxoid and streptokinase. An increase in the number of elevated antibody titres was seen after portocaval shunt operation. In no case was higher antibody titre associated with an increase in skin reactivity to the corresponding antigen. A number of significant titre changes to viral antigens were seen in the postoperative course without clinical evidence of the corresponding viral disease. These findings indicate that under certain circumstances antibody titre changes should be interpreted with caution.

The influence of the liver on antibody titres and γ -globulin levels has recently been discussed by several authors. The following causes of hypergammaglobulinaemia and high antibody titres have been postulated: 1) Non-specific altered globulin production in the diseased liver may give rise to non-specific elevated antibody titres (3, 4, 21). 2) Decreased activity of Kupffer cells due to damage of the liver and/or lowered blood flow through the liver could explain the rise of antibody titres because of larger amounts of circulating antigens not phagocytized by the liver (6, 30). 3) Lowered

immunosurveillance, resulting in increased growth and reduced clearance of microorganisms may increase the antigenic load presented to the antibody forming system (6, 19).

The present investigation has two purposes. 1) to follow antibody titres to common antigens in patients with liver cirrhosis undergoing portocaval shunt operations. 2) to estimate cell-mediated immunity (CMI) in these patients on the basis of skin tests.

PATIENTS

Sera were collected from patients with alcoholic and post-necrotic liver cirrhosis within an age range 25-67 years (9 males and 1 female). Diagnosis was confirmed by liver biopsy. All patients had suffered bleeding from oesophageal varices and were scheduled for surgery at the time the first sera were obtained. Twenty-one blood donors, aged 23-62 years (18 males and 3 females), served as controls.

Eight patients, aged 19-65 years (3 males and 5 females) undergoing cholecystectomy served as controls for skin test reactions as well as for antibody titres to diphtheria toxoid and *Candida albicans* precipitins. All controls had normal γ -globulin levels.

METHODS

The sera were examined for haemagglutination-inhibiting (HI) antibodies to rubella (12) and vaccinia viruses (16), and complement-fixing (CF) antibody to cytomegalo virus (CMV) (1).

Antistreptolysin O (AST) was determined according to Kalishak (15), antistreptococcal hyaluronidase (ASH) according to Faber (9), diphtheria toxoid (HI titre) according to Schöbel (23) and *Candida* precipitin were according to Axelsen et al. (2). Syphilis reactions included Wassermann (WR), Meinelike (MR) and Kahn tests (25). The "

Table 1 Comparison of the incidence of high antibody titres in cirrhotics before operation and in controls

	AST ≥160	ASH ≥7	Candi- da ≥3	Diph- theria ≥0.3	Typhoid-paratyphoid				Syphilis			CMV ≥12	Ru- bella ≥40	Vacci- nia ≥32	Total no of elevated titres
					PO	TO	PH	TH	Kahn	MR	WR				
Cirrhotics	3/10	2/10	4/10	6/10	2/10	0/10	0/10	0/10	0/10	2/10	0/10	2/10	7/10	8/10	36/140
Controls	0/21	0/21	1/8	0/8	0/13	0/13	0/13	0/13	0/13	0/13	0/10	1/14	5/21	2/13	9/194
P (%)	2.7			1.1									1.9	0.3	

Fisher's exact test.

was performed for *Salmonella typhi* O and H (TO TH) and *Salmonella paratyphi* O and H (PO PH) (8).

Skin tests were performed with 0.1 ml *Candida albicans* extract (1:1000) (Alergologisk Laboratorium, Copenhagen), 0.1 ml diphtheria toxoid II antigen (Statens Seruminstitut, 0.5 L/fml), 0.1 ml streptokinase equal to 10 IU (Behringwerke) and 0.1 ml purified tuberculin RT23 equal to 1 IU (Statens Seruminstitut, Copenhagen). The diameter of the induration following antigen injection was measured 48 hours after inoculation.

The left thigh was used for initial skin tests before operation, while the right was used for retesting after operation.

The antibody titrations other than rubella and vaccinia, were performed by Dr H. K. Andersen, Institute of Medical Microbiology Århus University, Dr N. H. Aschén, Protein Laboratory University of Copenhagen and the Diagnostic Bacteriology (the Serum & Vaccine, the Streptococcal and the Treponematoses Departments of the Statens Seruminstitut. The statistical calculations were performed by the Biostatistical Department of the Statens Seruminstitut.

RESULTS

Table I shows that rubella HI, vaccinia-HI, diphtheria antitoxin and AST titres are significantly higher in cirrhotics than in controls. In addition an

increased number of elevated titres against ASH, *Candida*, PO, MR and CMV are seen in these patients; the total number of elevated antibody titres being 36 of 140 as compared to 9 of 194 in the controls.

Table II shows the results of an antibody titre index calculation for the 10 cirrhotics approximately 1 month after portocaval shunt operation. The calculation is based upon determination of antibody titres to 9 different antigens. It will be seen that 8 of 10 patients had a positive titre index and none were negative. The result is significant ($p < 0.004$, Wilcoxon's sign test).

Table III shows an example of titre changes in a single patient after portocaval shunt operation. The patient showed a significant increase in rubella titre from 20 to 160 and an elevation of vaccinia titre from 32 to 256.

Fig. 1 shows the results of skin tests performed preoperatively with *Candida albicans*, diphtheria toxoid, streptokinase and tuberculin in the group of patients with liver cirrhosis and in the group with gallstones. Skin test reactions to the four antigens used were reduced in the liver cir-

Table II Titre index in liver cirrhosis one month after portocaval shunt operation
+1=Increase 0=unchanged, -1=decrease in titre

Antibody	Pat. no.										Antibody titre changes	
	1	2	3	4	5	6	7	8	9	10	Increases (n)	Decreases (n)
Rubella	-1	+1	-1	+1	0	0	0	0	+1	0	3	2
Vaccinia	-1	0	+1	+1	+1	0	0	-1	0	+1	4	2
CMV	0	+1	+1	+1	0	+1	+1	+1	+1	0	7	0
AST	+1	0	+1	-1	+1	+1	0	+1	+1	0	6	1
ASH	0	0	+1	-1	0	0	0	-1	0	0	1	2
MR	+1	0	0	+1	0	+1	+1	+1	0	-1	5	1
WR	0	0	0	+1	+1	0	0	+1	0	0	3	0
TH	0	0	0	0	0	0	+1	0	0	0	1	0
PO	0	0	0	-1	0	0	+1	0	0	0	1	1
Total index score	0	2	3	2	3	3	4	2	3	0		

Table III Antibody titre changes in patient 4 after portocaval shunt operation

No. of days before or after operation	Ra-betta	Vac chain	CMV
-8	20	32	4
+10	40	256	8
+17	160	64	16
+60	40	32	8

rhosis group compared to the gallstone group, though significantly only to diphtheria toxoid and streptokinase.

In Fig. 1 mean titres to diphtheria toxoid and *Candida albicans* and results of skin tests to these antigens pre- and postoperatively for 3 cirrhotic patients undergoing portocaval shunt operations are compared with the results obtained from 5 patients undergoing cholecystectomy. After portocaval shunt the mean antibody titres increased concomitantly with a decrease in the skin test reactions while no titre changes and a normal increase in induration were observed after cholecystectomy.

DISCUSSION

Our results indicate that elevated antibody titres to a number of common bacterial, fungal and viral antigens are found in patients with liver cirrhosis.

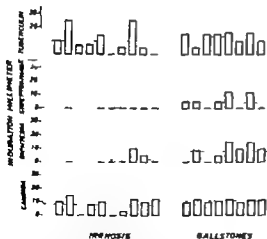


Fig. 1 Skin test reactivity to *Candida albicans*, diphtheria toxoid, streptokinase and tuberculin in patients with cirrhosis and patients with gallstones. — means induration, $p < 5\%$, - $p < 1\%$ (Wilcoxon's two sample test).

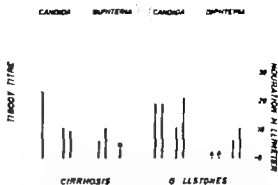


Fig. 2 Changes in antibody titre and skin reactivity to *Candida albicans* and diphtheria toxoid in patients undergoing portocaval shunt operation (mean of 3 patients) or cholecystectomy (mean of 5 patients). Antibody titre Δ pre- and Δ postoperatively, induration \circ pre- and \circ postoperatively.

After portocaval shunt these titres increased and in the postoperative course there were a number of titre changes to the antibodies in question. Depressed CMI is well recognized in many forms of liver diseases (10, 11, 17, 20). This study shows that skin reactivity to common antigens such as streptokinase and diphtheria toxoid may also be impaired. In organ transplanted patients repeated skin tests to tetanus toxoid failed to produce increased responses contrary to the findings in normal individuals (13). In the three cirrhotics skin tested twice there was a lack of booster effect, whereas none of the five cholecystectomized patients showed a reduced skin reaction to the postoperative skin test.

Circulating autoantibodies (7, 14, 32) and antibodies to bacterial (3, 4, 21, 30), viral (6) and certain dietary proteins (30) are reported to be increased in liver diseases. However, high levels of autoantibodies and antibodies to viruses and bacteria are also found under various conditions associated with impaired CMI (1, 26).

Recent publications (29, 31) have discussed the pronounced hyperglobulinaemia in liver diseases, and an increased amount of circulating antigens as well as an impaired T cell function are suggested as the causative mechanism. We would like to add the suggestion that raised plasma cortisol level due to protein deficient diet (24, 27) altered hormone turnover and cortisone treatment (5, 4) could lower an already impaired T cell suppressor function and further enhance antibody production (28). Our find-

ings of significant titre changes to viral antigens in the postoperative course may be explained as non-specific anamnestic responses or as a response to reactivated infections. Since diphtheria antitoxin pertains to an antigen other than a living microorganism a non-specific anamnestic response is likely at least in this case. The Kahn, TO and PH tests which are rather specific for the respective infections were negative in all portocaval patients as well as in controls. The raised antisalmonella agglutinins in 11 of 40 patients and in 9 of 20 patients with liver diseases reported from the USA (21) and Italy (3) respectively are not in accordance with our findings. The lower incidence of typhoid and paratyphoid fever in Denmark compared to these countries could however explain the discrepancy.

The increase in antibody titres after portocaval shunt is in accordance with the reported increase in serum globulins and antibody titres to *Escherichia coli* following portocaval shunt operation in both humans and rats (18, 22). Whether this is caused primarily by diminished blood flow through the liver or by the strain of major surgery remains to be determined. However we would like to suggest that the humoral responses to the operation are to some extent caused by suppression of the CMI leading to reactivation of intercurrent and latent infections.

REFERENCES

- Andersen H. K. & Spencer E. S. *Acta med scand* 186: 7 1969.
- Axelsson N. H., Kirkpatrick, C. H. & Bockley R. H. *Chn. exp Immunol* in press 1974.
- Bianchi P. A., Porro G. B., Attili L. & Razzi, T. 9. Congrès internat. de Gastro-entéro 234 c 1972.
- Bjorneboe, M., Prytz, H. & Ørskov F. *Lancet* 1 58 1972.
- Chase J. H., White A. & Dougherty T. F. *J Immunol* 52: 101 1946.
- Choss O., Haukenes, O., Gjone E. & Blomhoff J. P. *Lancet* 2, 1202, 1971.
- Doniach, D., Roitt, I. M., Walker J. G. & Sherlock, S. *Clin exp Immunol* 1 237 1966.
- Faarup C. Diss. Munksgaard Copenhagen 1937.
- Faber V. Diss. University of Copenhagen 1955.
- Fox R. A., Dudley F. J. & Sherlock, S. *Clin. exp. Immunol* 14 473 1973.
- Gardner R. J., Hart, J. T., Sutton, W. R. & Preston, F. W. *Surg. Forum* 12, 167 1961.
- Halonon P. E., Ryan J. M. & Stewart, J. A. *Proc Soc exp Biol (N.Y.)* 125 16., 1967.
- Johansen K. S., Johansen, T. S. & Starzl T. E. *Scand J Immunol* in press 1974.
- Johnson, G. D., Holborow E. J. & Glynn, L. E. *Lancet* 2, 416 1966.
- Kalbak, K. Diss. Munksgaard Copenhagen 1942.
- Kempe C. H. & St. Vincent, L. *Amer Publ Hlth. Ass* 111 New York 1964.
- Mella, B. & Lang D. J. *Science* 155 80 1967.
- Meyers, O. L. & Korman M. *Clt. after Trigr* D. R. & Wright, R. *Lancet* 2, 1494 1973.
- Papageorgiou N. S. & Glade P. R. *New Engl. J. Med.* 285 581 1971.
- Pappas, A., Phippen, R., Pütz, Th. & Scherren, P. *G. Klin. Woch* 22, 1362 1970.
- Protel R. L., Soloway R. D., Martin, W. J., Schoenfeld L. J. & Summerskill W. H. *J. Laecet* 2, 330 1971.
- Prytz, H., Bjorneboe M., Stehr-Johansen T. & Ørskov F. *Acta med scand* 196 109 1974.
- Scheibel I. *Acta path. microbiol. scand.* 39 455 1956.
- Schoonland M. M., Shenley B. C., Loening W. E. K., Parent, M. A. & Coovadia H. M. *Lancet* 2, 435 1972.
- Smith H. *Brit. J. veter. Dr.* 27 23 1951.
- Smith M. G. M., Williams, R., Doniach, D. & Calne R. Y. *Lancet* 2, 1006, 1971.
- Snythe P. M., Schoonland M., Breton-Stiles H. D., Coovadia, H. M., Grace H. J., Loening, W. E. K., Maf yane A., Parent, M. A. & Vos, G. H. *Lancet* 2, 939 1971.
- Tada, T., Taniguchi, M. & Okumura, K. O. *J Immunol* 106-1012, 1971.
- Thomas, H. C., McSweeney R. N. M. & White R. G. *Lancet* 1 1288 1973.
- Triger D. R., Alp H. H. & Wright, R. *Lancet* 1 60, 1972.
- Triger D. R. & Wright, R. *Lancet* 1 1494 1973.
- Whittingham S., Mackay L. R. & Irwin, J. *Lancet* 1 1333 1966.

LIVER IMPAIRMENT DURING CHRONIC HEMODIALYSIS AND AFTER RENAL TRANSPLANTATION

Victor Nielsen Ebbm Clausen and Leo Ranck

From Medical Departments P and A Rigshospitalet, Copenhagen Denmark

Abstract Liver impairment has been evaluated in consecutive series of 79 patients with chronic renal failure of whom 23 were treated with hemodialysis alone and 37 with hemodialysis and renal transplantation alone. In half of the chronic hemodialysis patients and half of the patients receiving renal allograft elevation of serum alanine aminotransferases was observed for a shorter or longer period during the study. In half of these cases from both groups the clinical course, laboratory data and liver histology were consistent with virus hepatitis and four patients died from fulminant hepatic failure. In the other half of the patients with elevated transaminases, this was either asymptomatic and unexplained or due to other causes such as septicemia or urinary leakage. Liver biopsy showed unspecific changes. Renal transplantation was not performed in patients suffering from virus hepatitis but 12 of the 37 patients who received renal allograft had elevated aminotransferases at the time of transplantation. In seven of them a marked increase in aminotransferase was observed postoperatively but none developed clinical sign of liver disease. It is concluded that elevated aminotransferase activity per se is no contraindication to surgical procedures, including renal transplantation, in these patients. However liver biopsy should be performed to detect possible liver disease.

Liver impairment is often seen in patients treated with chronic hemodialysis and in patients who have received a renal allograft (4 8 9 10 11 13 14 20 23 24). Virus hepatitis has most often been incriminated. Of the 79 patients reported here half of those treated with chronic hemodialysis and half of those treated with a renal allograft showed elevation of alanine aminotransferase activity in serum. As surgical procedures may be fatal in patients with virus hepatitis it is important to know whether elevation of aminotransferases in a patient who is going to receive a renal transplant is due to hepatitis. Likewise elevation of aminotransferases in a patient receiving immunosuppressive treatment after transplantation represents a problem as both toxic liver

damage and virus hepatitis may be the cause. The purpose of the present work is to evaluate the possible causes of elevation of aminotransferases in patients treated with chronic hemodialysis and renal transplantation.

MATERIAL AND METHODS

The material consists of consecutive series of 79 patients, 23 of whom were treated with hemodialysis alone (May 1963 - May 1970), 37 with hemodialysis and renal transplantation, and 19 with allotransplantation alone (Jan. 1968 - May 1970). Among the latter 11 patients had previously been treated with hemodialysis in other centres.

The material is divided into hemodialysis patients ($n=60$) and patients followed after renal transplantation ($n=56$). 37 patients were studied both during hemodialysis and after transplantation, and are thus included in both groups.

The following liver tests were performed at least four times per month: serum alanine aminotransferase, prothrombin-proconvertin and electrophoresis of serum proteins. From Oct. 1968 cytomegalic virus antibody titre in serum and from Nov. 1969 Hb-s antigen (27) were determined in all patients. Liver biopsies were performed with the Menghini needle. Liver histology either from biopsy or autopsy was assessed in 30 patients.

Acute hepatitis was suspected in patients with sudden rise in alanine aminotransferases concomitantly with clinical symptoms consistent with virus hepatitis. The diagnosis was verified by histology in half of the cases. Clinically, biochemically and histologically the chronic hemodialysis patients and the patients who had received renal allograft could be divided into the following five groups: I) no clinical or biochemical evidence of liver impairment, II) elevated alanine aminotransferases but no clinical evidence of liver disease, III) acute hepatitis, IV) chronic hepatitis, V) elevated alanine aminotransferases possibly due to other causes than virus hepatitis.

Hemodialysis technique

Three types of dialysers have been used: Skegg-Leonards (25) until April 1964; Kii[®] (17) until March 1969 and thereafter Gambro disposable dialysers (1).

Table 1 Hemodialysis material

No. of pts.		Observation period (d.)	Hemodialysis* (n)	Blood transfusions* (n)	Liver biopsies or autopsies	Hepatic precoma or coma	Deaths from liver failure	Renal allografts
29	Without liver impairment	163 (7-540)	32 (2-242)	41 (4-107)	6			23
9	Asymptomatic alanine aminotransferase elevation	669 (90-1 980)	146 (7-520)	112 (7-307)	2			7
5	Anicteric hepatitis	624 (60-1 280)	162 (17-342)	96 (17-242)	3			3
10	Icteric hepatitis	254 (30-490)	45 (5-97)	40 (12-80)	7	5	3	1
7	Other causes	514 (30-1 890)	148 (15-513)	190 (48-491)	4			3
60	Total				22	5	3	37

Average, range within parentheses.

The first two types of dialysers were used in combination with pumps constructed by Ole Deth, Denmark, or a Travco pump on the blood side. The Gambro dialysers have been used in connection with DDS consoles (6, 7). In this system contamination of the equipment can be avoided (7). (Luprophane® pT 150 membranes have been used in the dialysers. Membranes coated with the K₂ and the Leonard's dialysers were boiled for 90 min and in Rodalon® 1%, phenoxethyl 1% or chloramine

3% solution before use. Prior to use of the DDS consoles the machinery was perfused with the same solutions and once a week with a 3% acetic acid solution. The tubes were gamma-sterilized PVC for medical use.

The patients were dialysed either through an internal Cremo-Brescia fistula or through an external shunt a.m. Stribner-Quinton. All patients were dialysed in the hospital for 10-16 hours twice a week. On an average the serum creatinine concentration fell from 12.4 to 4.4 mg/100 ml, and BUN from 127 to 19 mg/100 ml. The patients were hospitalized only in connection with shunt problems or intercurrent diseases. Most of the patients were gainfully employed during the treatment.

Renal transplantation

Patients receiving a renal allograft were anaesthetized with barbitalate-sodiumethonamchloride N_2O-O_2 -cyclopropane. Thirty patients were treated with extracorporeal irrigation of the blood (28) before transplantation. After transplantation the immunosuppressive treatment consisted of prednisone 40-60 mg daily and azathioprine (Imuran®), 5 mg/kg b.wt. on the first day and thereafter approximately 2 mg/kg b.wt. daily.

RESULTS

Hemodialysis material

The average observation period, number of hemodialyses and blood transfusions are given in Table 1. The fewest number of hemodialyses and blood transfusions are seen among patients without liver impairment and among patients with icteric hepatitis. The differences, however, are not statistically significant.

In 29 patients (group I) no liver impairment was observed during hemodialysis.

In nine patients (group II) elevated alanine aminotransferases were observed but no symptoms or clinical sign of liver disease was present. Characteristically the enzyme elevation was small ($2-4 \mu\text{mol/h/ml}$) and lasted for 2-4 months, except for one case in whom it persisted for one year. Hb-antigen test was negative in eight patients and in one patient it was not performed. Liver biopsy was performed in two patients and the liver histology was normal in both.

Five patients (group III) had anicteric hepatitis. The diagnosis was based on a typical clinical course and typical course of aminotransferase elevation. Three of the diagnoses were confirmed by liver biopsy. Hb-antigen was investigated during the acute phase of the disease in one patient and in four

Table II Results in hemodialysis patients undergoing renal transplantation (RAT)

No. of pts.		During hemodialysis		After RAT				
		Elevated alanine aminotransferase at the time of RAT	Peak enzyme elevation 1 mo. after RAT	No liver impairment	Asymptomatic alanine aminotransferase elevation	Azotemic hepatitis	Icteric hepatitis	Other causes
23	Without liver impairment			12	4	4		1
7	Asymptomatic alanine aminotransferase elevation	7	3	2	4	1		
3	Azotemic hepatitis	3	3			3		
1	Icteric hepatitis	1	0		1			
3	Other causes	1	1	1				2
37	Total	12	7	15	9	8	2	3

patients after the acute disease. The antigen was present in three patients. In one of them two liver biopsies performed with an interval of six months showed chronic aggressive hepatitis.

Ten patients (group IV) had icteric hepatitis, six of whom had typical virus hepatitis and four cholestatic hepatitis with high alkaline phosphatases and high serum bilirubin values. Evidence of drug-induced hepatitis was not found, and none of them received azathioprine or corticosteroids.

The liver biopsies were almost identical showing marked centrilobular cholestasis, few liver cell necroses and practically no cellular infiltration. In the other patients liver histology was typical of virus hepatitis ranging from spotty to total liver cell necroses.

Five patients lapsed into hepatic precoma or coma, and three of them died from hepatic failure. Hb-antigen was investigated during the acute phase of the disease in five of the patients and at various times after the disease in two; all were negative.

Group V comprises seven patients, all without jaundice. Elevated alanine aminotransferases were assumed to be due to bacterial sepsis in two patients, crush syndrome with anuria in two, iron contamination of the dialysis water in one, cardiac arrest in one, and severe hemosiderosis of the liver with focal necroses of the liver cells in one patient.

Cytomegalic virus titres showed no significant rise during the investigation period in any of the patients examined.

Renal allotransplantation material

Thirty-seven of the hemodialysis patients received a renal allograft (Table II). Eleven of 23 patients in group I developed liver impairment, in six due to virus hepatitis. One patient with hepatitis died three months after the renal transplantation in fulminant hepatic failure.

All the patients in groups II, III and IV had elevated alanine aminotransferases at the time of transplantation and in six a marked increase was observed during the first month after transplantation. None of them had jaundice or other clinical signs of liver disease. The patients with virus hepatitis during hemodialysis (3 in group III and 1 in group IV (Table I)) underwent renal transplantation at a time when clinical signs of hepatitis were absent. The shortest period between hepatitis and operation was one month.

In Table III patients treated with renal allografts are divided into the same five groups as the hemodialysis patients and the corresponding figures are given. In conformity with the hemodialysis material the fewest number of hemodialyses and blood transfusions were seen among patients without liver impairment and among patients with icteric hepatitis.

In 28 patients (group I) no liver impairment was observed. Asymptomatic elevation of alanine aminotransferases was seen in nine patients (group II). Liver biopsy was performed in two patients. One biopsy showed moderate to marked fatty in-

Table III Renal allotransplantation (RAT) material

No. of pts.		Observation period after RAT* (d)	Hemodialysis* (n)	Blood transfusions* (n)	Liver biopsies or autopsies	Hepatic precoma or coma	Deaths from liver failure	Treated with hemodialysis
28	Without liver impairment	175 (30-720)	54 (0-533)	38 (1-277)				23
9	Asymptomatic alanine aminotransferase elevation	340 (30-854)	66 (5-183)	76 (7-463)	2			7
III	Anicteric hepatitis	380 (215-610)	103 (0-342)	94 (12-242)	2			1
4	Icteric hepatitis	189 (120-335)	63 (0-200)	26 (16-38)	3	2	1	1
5	Other causes	231 (90-396)	133 (9-513)	116 (8-496)	1			3
56	Total				8	2	1	37

Average, range within parentheses

filtration and the other a few liver cells with cloudy swelling. In this patient Hb-antigen test was positive. Three other patients in this group were Hb-antigen negative.

Anicteric hepatitis was suspected in ten patients (group III) and the diagnosis was verified in two by biopsy. Hb-antigen test was positive in eight and negative in two patients.

Icteric hepatitis was seen in four patients (group V), two of whom lapsed into hepatic precoma or a. As previously mentioned one of them died hepatic failure. Hb-antigen test was positive in one and negative in two patients. The antigen was not determined at the time when the patient with fulminant hepatitis died.

Five patients (group V) were assumed to have elevated alanine aminotransferases due to the following causes: one had polycystic kidneys and cysts in the liver (verified at biopsy); three developed alanine aminotransferase elevation in connection with urinary leakage and urinary tract infection; and one had marked hemosiderosis in the liver (the same patient as in the hemodialysis material). Hb-antigen test was negative in two of these patients. Cy to megalic virus titres did not rise significantly in any of the patients treated with renal allotransplantation.

Staff

The staff was checked twice monthly for elevation of alanine aminotransferases. Four members devel-

oped hepatitis during the investigation period. All were jaundiced and the clinical course was mild. All these cases appeared before Hb-antigen test could be carried out, but later investigations have revealed no Hb-antigen positive cases among the staff (27).

DISCUSSION

In the present material 50% of the chronic hemodialysis patients and 50% of those treated with renal transplantation had elevated alanine aminotransferases for shorter or longer periods. Outbreak of hepatitis in dialysis centres has been a serious complication in the treatment of chronic renal failure (4, 8, 9, 10, 11, 13, 14, 20, 23, 24), and some centres have been forced to interrupt hemodialysis treatment and renal transplantation for this reason (18). In this centre hepatitis has been observed throughout the 7-year study period, but no regular epidemic outbreaks have occurred. The increase in the number of patients with hepatitis parallels the number of patients treated with chronic hemodialysis. The mortality from hepatitis was 13%. No case of postnecrotic cirrhosis was seen, but one patient developed chronic aggressive hepatitis. None of the patients received a renal graft during the acute phase of hepatitis, but 12 had elevated aminotransferases at the time of transplantation. In seven of them a short-lasting marked increase in serum enzymes occurred after the transplantation, but none became jaundiced or died. This indicates

that the elevation of alanine aminotransferases is not a contraindication for performing renal transplantation. Death from hepatic failure after renal transplantation has been reported (5-13) and one of our patients died from hepatitis three months after transplantation. This patient had normal alanine aminotransferases and no evidence of hepatic dysfunction at the time of transplantation.

As pointed out by London et al (19) serum alanine aminotransferase is probably the most valuable test for liver disease in patients treated with chronic hemodialysis. Many patients receive anticoagulants and the prothrombin concentration is therefore diagnostically useless. Alkaline phosphatases may be elevated due to osteomalacia and/or secondary hyperparathyroidism and albumin may be low due to restricted protein intake. Bromsulphalein retention may be of greater value but was not performed in all our patients. Many authors have used elevated alanine aminotransferases as a conclusive criterion of virus hepatitis in patients treated with chronic hemodialysis and renal transplantation. We interpret our data so that only half of our patients with elevated alanine aminotransferases suffered from virus hepatitis.

As mentioned by Mattenheimer et al (21) and Bergman et al (3), however several causes of the hepatic involvement are possible in these patients. Cytomegalic virus infection has been reported during chronic hemodialysis and after renal transplantation (2-9), but cytomegalic virus titres did not rise significantly in any of our patients. Different drugs such as tranquilizers, analgetics, antibiotics, anticoagulants and immunosuppressive drugs especially azathioprine and corticosteroids, have been given to patients in the present study. Liver impairment due to most of these drugs is well known but no evidence of drug-induced liver damage was found in the present series and especially no case of azathioprine cholestasis was seen (26). Liver damage in connection with extracorporeal irradiation of the blood has not been described but has been reported due to split products (esters of fatty acid) from the gamma-sterilized PVC tubes (15-22) and we cannot exclude that this was the cause in some of our patients. Enzyme elevations and abdominal discomfort, however, were not seen in immediate connection with the hemodialyses. Our patients have received a diet containing at least 40-50 g protein per day which should exclude malnutrition as a cause of liver impairment (29). The cause of asymptomatic

enzyme elevations in our material remains unknown but some of these patients might have had an asymptomatic and slight hepatitis which however appears to be of no clinical significance. Liver biopsy is valuable in assessing liver disease in these patients and the risk of bleeding following liver biopsy is minimal if the biopsy is taken about 12 hours after stopping hemodialysis, and if protamine sulphate is given at the end of the hemodialysis. No complication after liver biopsy performed under these conditions was seen.

Hb-antigen determinations were performed only during the last 6 months of this investigation. It is striking however that none of the chronic hemodialysis patients were Hb-antigen positive. Among patients who had received a renal allograft, 11 were Hb-antigen positive but it is not known whether they were positive or negative before the transplantation. In this centre fortunately no serious cases of hepatitis occurred among the staff.

REFERENCES

1. Alwall, N. A new disposable artificial kidney: experimental and clinical experience. *Proc Europ Dial Transp Ass.* 5: 18 1968.
2. Armstrong, D., Balakrishnan, E. L., Steger, L., Yu, B. & Stenzel, K. H. Cytomegalovirus infections with viremia following renal transplantation. *Arch intern Med.* 127: 111 1971.
3. Bergman, L. A., Thomas, W., Reddy, C. R., Ellison, M. R., Smith, E. C. & Dunea, G. Nonviral hepatitis in patients maintained by long-term dialysis. *Arch intern Med.* 130: 96, 1972.
4. Briggs, W. A., Lazarus, J. M., Birch, A. G., Hampers, C. L., Hager, E. B. & Merrill, J. P. Hepatitis affecting hemodialysis and transplant patients. *Arch intern Med.* 132: 21 1973.
5. Collin, L. G., Bergström, K., Fjorvick, C., Magnusson, O., Nordström, H., Wehle, B. & Werner, B. Leverdysfunktion samband med njur transplantation. *Nord. Med.* 46: 1567 1968.
6. Dawkins, S. G., Boe, C. & Andreasen, M. M. A new hemodialysis console. I. *Acta med. scand.* 193: 373 1973.
7. — A new hemodialysis console. II. *Acta med. scand.* 193: 379 1973.
8. Editorial: Hepatitis virus and renal dialysis. *Lancet* 2: 989 1969.
9. Evans, H. B., Mallard, H. R. & Herbertson, B. M. Hepatic dysfunction associated with renal transplantation. *Lancet* 2: 929 1968.
10. Fredman, E. A. & Thomson, O. E. Hepatitis complicating chronic hemodialysis. *Lancet* 2: 675 1966.
11. Garbade, R. A., Forrest, J. N., Bryan, J. A., Hanson, B. F. & Dynesius, W. E. Hemodialysis-associated hepatitis. *J.A.M.A.* 225: 384 1973.

12. Hennekens C H. Hemodialysis-associated hepatitis. *J.A.M.A.* 225: 407 1973.
13. Ireland, P., Rashid, A., Lichtenberg, F., Cavallo T. & Merrill J. P.. Liver disease in kidney transplant patients receiving azathioprine. *Arch. Intern. Med.* 132: 29 1973.
14. Ivey K. J. & Clifton, J. A.. Liver disease in patients treated with chronic hemodialysis. *Gastroenterology* 4: 59 1970.
15. Jaeger R. J. & Rubin, R. J. Plasticizers from P.V.C. *Lancet* 2: 778 1970.
16. Jones, P. O., Goldsmith, H. J., Wright, F. K., Roberts, C. & Watson, D. C. Viral hepatitis—A staff hazard in dialysis units. *Lancet* 1: 835 1967.
17. KEL, F. Development of parallel flow artificial kidney in plastics. *Acta chir. scand. Suppl.* 253: 142 1960.
18. Leading article: Hepatitis in dialysis units. *Brit. med. J.* 4: 255 1970.
19. London, W. T., Di Figlia, M., Setnick, A. I. & Blumberg, B. S. An epidemic of hepatitis in a chronic-hemodialysis unit. *New Engl. J. Med.* 281: 571 1969.
20. Marmion, B. F. & Tonkin, R. W.. Control of hepatitis in dialysis units. *Brit. med. Bull.* 28: 169 1972.
21. Matthesheimer H., Friedel, R. & Schwartz, F. D. Hepatopathy of chronic hemodialysis in the absence of hepatitis. *Gastroenterology* 58: 310 1970.
22. Neergaard J., Nielsen, B., Faurby V., Christensen, D. H. & Nielsen, O. F.. Anvendelse af polyvinyl-klorid og forekomsten af hepatitis. *Ugeskr. Læg.* 133: 354 1971.
23. Polakoff S., Coswart, Y. E. & Tillert, H. E. Hepatitis in dialysis units in the United Kingdom. *Brit. med. J.* 8: 94 1972.
24. Ringertz, O. & Nyström, B. Viral hepatitis in connection with hemodialysis and kidney transplantation. *Scand. J. Urol. Nephrol.* 1: 192, 1967.
25. Skogga, L. T., Leonards, J. R. & Heister C. R.. Artificial kidney II. Construction and operation of improved continuous dialyzer. *Proc. Soc. exper. Biol. (N.Y.)* 72: 539 1949.
26. Sparberg, M., Simon, N. & Greco, F. D. Intrahepatic cholestasis due to azathioprine. *Gastroenterology* 57: 439 1969.
27. Steinest, I. & Skinhøj, P. Hepatitis associated antigen: Elimination from a dialysis unit and persistence in renal transplant recipients. *Acta path. microbiol. scand. Sect. B* 79: 725 1971.
28. Wecke E., Andersen, V., Friesleben-Sørensen, S. & Bahr, B. Extracorporeal irradiation of the blood as immunosuppressive treatment in renal transplantation. *Acta med. scand.* 187: 183 1970.
29. Young, G. A. & Parsons, F. M. Is hepatitis in chronic renal failure associated with impaired hepatic function? *Lancet* 2: 1130, 1970.

CLINICAL LISTERIOSIS IN RENAL ALLOTRANSPLANTATION

Erik Christensen

From Medical Department P, Division of Nephrology
Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Abstract Two cases of *Listeria monocytogenes meningitis* among 212 kidney transplanted patients (total of 339 patient years of observation) under immunosuppression with azathioprine and prednisone are presented. Both cases developed shortly after an increase of the immunosuppression. The first case appeared in a 27-year-old man 5 days after minor increase of the azathioprine dose from 75 to 100 mg/day. The course was relatively mild, and the patient was cured by tetracycline. The second case appeared in a 52-year-old woman 5 days after massive increase of the steroid dose and administration of moderate azathioprine dose failed to revert rejection of the graft. This case had a fulminant course and was complicated by *Listeria* sepsis with hemolysis, pronounced oliguria and thrombocytopenia leading to fatal internal bleedings, primarily in the brain. Considering the poor prognosis of this complication it is suggested that cytotoxic drugs are temporarily discontinued and the steroid dose reduced at the height of the infection.

The rather common infection with *Listeria monocytogenes* is generally asymptomatic in healthy untreated individuals (1).

Clinical listeriosis is usually found in individuals with lowered resistance relating to malignancy (leukemia, lymphoma, Hodgkin's disease), diabetes mellitus, cirrhosis of the liver and/or medication with cytotoxic drugs or steroid hormones (1, 2, 4, 5, 11, 12, 13). A few cases have, moreover, been reported in immunosuppressed patients after renal allotransplantation (6, 7, 11).

This paper describes cases of *Listeria meningitis* among 21 immunosuppressed kidney transplanted persons, who have lived on an average 1.6 years after grafting.

CASE REPORTS

Case 1

A 27-year-old man with chronic glomerulonephritis received kidney transplant from his father (HL-A mismatch in one locus). Immunosuppression with prednisone and

azathioprine was started and after 4 months the patient was discharged with 4-hour creatinine clearance of 60-70 ml/min, no proteinuria and normal BP. At this time he received prednisone, 30 mg/day and azathioprine, 75 mg/day.

Five months after the transplantation the patient was readmitted on Jan. 13, 1969 after days of intense, diffuse headache, pains in the back and the neck, slight dysuria, but no diarrhea. On Jan. 9 the azathioprine dose had been increased from 75 to 100 mg/day. On admission the patient was relatively unaffected. Physical examination revealed only temperature of 38.7°C and slight tenderness, but no stiffness of the neck. Hb 15.1 g/100 ml, WBC 15 000/mm³ with 89% polymorphonuclear leukocytes, 7% lymphocytes, 2% monocytes and 2% neutrophil myelocytes, thrombocytes 97 000/mm³. Blood cultures were negative. Urinalysis revealed 5-8 leukocytes, 5-7 erythrocytes and bacteria. As urine cultures, performed on Jan. 9 in the Outpatient Service, had grown more than 10⁶ *Klebsiella pneumoniae* per ml, treatment with tetracycline was started. However, during the day the temperature rose to 40.1°C.

On Jan. 11 stiffness of the neck and ataxias to the left was noted. Lumbar puncture was unsuccessful due to artificial bleeding. On Jan. 15 a new lumbar puncture revealed clear cerebrospinal fluid under normal pressure. Analysis gave WBC of 118/mm³ with 70% mononuclear and 30% polymuclear cells. Protein was 90 mg/100 ml and glucose 54 mg/100 ml (blood glucose 137 mg/100 ml). Microscopy and culture of the spinal fluid revealed no microorganisms. EEG was normal. Urine culture from the day of admission had now revealed significant growth of *Klebsiella pneumoniae* resistant to tetracycline, which therefore was discontinued after administration of total dose of 1.75 g within 36 hours. Treatment with colistin 4 million U/day and cephalothin 4 g/day was started.

On January 16 the temperature decreased, but pains of the left abducent nerve had developed. On the next day the temperature rose again, but blood cultures remained negative. EEG was normal.

On January 18 a new lumbar puncture revealed slightly cloudy spinal fluid under pressure of 470 mm H₂O. The WBC was 400/mm³ with 70% mononuclear and 30% polymuclear cells. Protein was 99 mg/100 ml and glucose 42 mg/100 ml (blood glucose 118 mg/100 ml). Microscopy and cultures of the spinal fluid revealed no microorganisms.

On Jan. 20 the EEG was markedly abnormal with

12. Hennekens C. H. Hemodialysis-associated hepatitis. *J.A.M.A.* 225 407 1973
13. Ireland, P., Rashid A., Lichtenberg, F. Cavallo T. & Merrill J. P. Liver disease in kidney transplant patients receiving azathioprine. *Arch. Intern. Med.* 132, 29 1973
14. Ivey K. J. & Clifton, J. A. Liver disease in patients treated with chronic hemodialysis. *Gastroenterology* 4 59 1970
15. Jaeger R. J. & Rubin R. J. Plasticisers from P.V.C. *Lancet* 2: 778 1970.
16. Jones, P. D. Goldsmith, H. J. Wright, P. K. Roberts, C. & Watson, D. C. Viral hepatitis—A staff hazard in dialysis units. *Lancet* i 835 1967
17. KEl, F. Development of a parallel flow artificial kidney in plastics. *Acta chir. scand. Suppl.* 253 142 1960
18. Leading article: Hepatitis in dialysis units. *Brit. med. J.* 4 255 1970
19. London, W. T. Da Figlia, M. Sutnick, A. I. & Blumberg, M. S. An epidemic of hepatitis in chronic-hemodialysis unit. *New Engl. J. Med.* 281 571 1969
20. Marmion B. P. & Tonkin R. W. Control of hepatitis in dialysis units. *Brit. med. Bull.* 28 169 1972.
21. Matzenheimer H. Friedel, R. & Schwartz, F. H. Hepatopathy of chronic hemodialysis in the absence of hepatitis. *Gastroenterology* 58, 310 1970.
22. Neergaard J. Nielsen, B. Faurby V. Christensen, D. H. & Nielsen O. F. Anvendelse af polyvinylklorid og forekomsten af hepatitis. *Ugeskr. Læg.* 133 354 1971
23. Polakoff S., Cossart Y. E. & Tibert H. E. Hepatitis in dialysis units in the United Kingdom. *Brit. med. J.* 3, 94 1972.
24. Ringertz, O. & Nystrom B. Viral hepatitis in connection with hemodialysis and kidney transplantation. *Scand. J. Urol. Nephrol.* 1 192, 1967
25. Skeggs L. T. Leonaards, J. R. & Heister C. R. Artificial kidney II. Construction and operation of improved continuous dialyzer. *Proc. Soc. exper. Biol. (N.Y.)* 72, 539 1949
26. Sparberg, M. Simon, N. & Greco F. D. Intrahepatic cholestasis due to azathioprine. *Gastroenterology* 57 439 1969
27. Steinoss, I. & Skimhej P. Hepatitis associated antigen Elimination from a dialysis unit and persistence in renal transplant recipients. *Acta path. microbiol. scand. Sect. B* 79: 725 1971
28. Worske E. Andersen, V. Friesleben-Sørensen, S. & Bahr B. Extracorporeal irradiation of the blood as immunosuppressive treatment in renal transplantation. *Acta med. scand.* 187 183 1970.
29. Young, G. A. & Parsons, F. M. Is hepatitis in chronic renal failure associated with impaired hepatic function? *Lancet* 2, 1130, 1970.

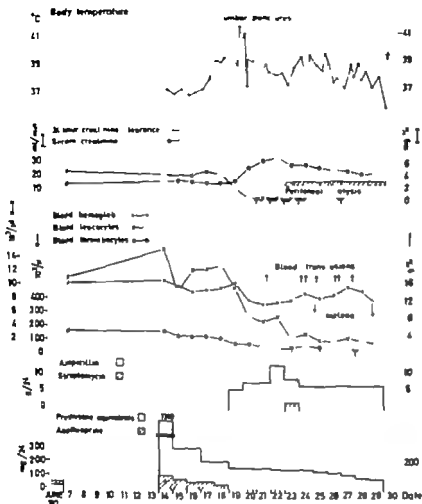


Fig 2 Temperature, kidney function, Hb leucocytes, thrombocytes, antibiotic treatment and immunosuppression in case 2

serum creatinine 3.2 mg/100 ml. Urine cultures were negative. WBC was 15 000/mm³, thrombocytes 154 000/mm³ and Hb 17 g/100 ml. Rejection of the graft was suspected and 1 g methylprednisolone (Sotamedrone®) was administered followed on the next day by 300 mg prednisone which was thereafter gradually reduced. Azathioprine, which, due to thrombocytopenia, had been discontinued for a week, was now given a dose of 100 mg/day being gradually reduced over the next days. Hereby the kidney function improved little.

On June 18 the temperature rose and at the same time the BP temporarily fell to 105/65 mmHg. Simultaneously the patient got headache and pain in the back. The neck and back were both supple. On June 19 the patient had a stiff neck and back.

Lumbar puncture revealed slightly cloudy spinal fluid under pressure of 400 mm H₂O. It contained numerous polymorphs and some mononuclear leucocytes, elevated protein and lowered (<30 mg/100 ml) glucose (temporarily only determined semiquantitatively). Numerous Listeria monocytogenes were demonstrated by microscopy and later cultures of the spinal fluid. Cul-

tures of the blood grew *L. monocytogenes* in 7 of 7 glasses.

Treatment with ampicillin, 6 g/day i.v. was started. However the patient became increasingly confused, stuporous and eventually unconscious, the temperature rose to 41°C the BP fell temporarily to 105/70 mmHg and the urine production decreased permanently to about 5 ml/min. Hb fell from 16 to 12.1 g/100 ml and to 11.0 g/100 ml on the next 2 days, serum bilirubin rose to 1.3 mg/100 ml and plasma Hb was 6.8 mg/100 ml. The leucocytes and the thrombocytes decreased permanently to very low values in spite of the discontinuation of azathioprine. The bleeding and coagulation times, which were normal on admission, were now >15 min and 13 min, respectively. The prothrombin time was normal and did not increase. Several emphysemata and petechiae appeared on the skin. Serum GPT which on admission was 22 U/l rose to 250 U/l serum LDH was now also elevated (temporarily no isoenzyme determination was done).

On June 22 a new lumbar puncture revealed xanthochromic spinal fluid containing 336 WBC/mm³ with 80% mononuclear and 20% polymorphous cells. Protein

was 234 mg/100 ml and glucose was normal. Some *L. monocytogenes* were found by microscopy and later in cultures of the spinal fluid. Ampicillin was temporarily increased to 12 g/day and 1 g streptomycin was given.

Due to the reduced kidney function, peritoneal dialysis was started. However the condition became worse a grand mal seizure developed followed by cardiac arrest. Both were relieved by relevant treatment. The ventilation now had to be assisted by a respirator. Various cardiac arrhythmias were treated effectively with antiarrhythmic drugs.

During the following days repeated universal epileptic seizures developed, BP fell and increasing pneumonic infiltrations evolved (growth of *Klebsiella oxytoca* and yeast-like fungi). Relevant treatment was not effective. Urine cultures remained negative. On June 23 the patient had pneumonia and to spite of blood transfusions and other supportive treatment she died on June 30.

At the bacteriologic autopsy numerous *Klebsiella oxytoca* were cultured from the heart, the lungs, the spleen, and the liver. No *Listeria monocytogenes* or fungi were cultured. The heart was enlarged. In the posterior wall of the left ventricle a big 10-12-day-old infarction was found. The myocardium was diffusely fibrous. The lungs, especially the lower lobes, contained numerous bronchopneumonic infiltrations. The stomach, the intestines and the colon contained much blood and the mucous membranes were labiled with blood. In the stomach more acute ulcerations were found. The kidney graft was moderately oedematous and pale, and on the surface two 10-14-day-old infarctions were seen. All anastomoses were sufficient without any reaction. Microscopy revealed chronic vascular rejection. Acute tubular necrosis could not be ruled out because of pronounced autolysis.

The autopsy of the brain revealed a considerable amount of blood in the subarachnoid space and numerous massive hematomas measuring up to 4 cm in diameter were found in the cerebrum and the cerebellum. No pus was seen in the arachnoid, and the meninges were not thickened.

Microscopically the leptomeninges presented slight patchy perivascular inflammatory reaction and a moderate bleeding beneath the arachnoid. In the white substance marked perivascular edema was present. No macrophagocytosis were demonstrated in Gram-stained sections.

DISCUSSION

Neither of the patients had a history of contact with animals or ill people nor had they any symptom or sign of gastrointestinal affection, which is supposed to promote the intrusion of *Listeria monocytogenes* into the organism (1).

Both cases were preceded by an increase in the immunosuppression, which may have activated latent, subclinical infections with *L. monocytogenes*.

In case 1 stiffness of the neck became apparent 5 days after an increase of the azathioprine dose from 75 to 100 mg/day. The prednisone dose of 30 mg/day

was constant throughout the course. No *L. monocytogenes* were demonstrated in the blood. The bacteriologic diagnosis was established rather late, as a concomitant urinary tract infection was treated for 36 hours with tetracycline which effectively suppresses the growth of *L. monocytogenes*. Not until tetracycline had been discontinued for 6 days was it possible to demonstrate *L. monocytogenes* in cultures of the spinal fluid. Resumed treatment with tetracycline was fully effective.

In case 2 stiffness of the neck appeared 5 days after a massive increase in the steroid dose and administration of a moderate azathioprine dose (Fig. 2) in an attempt to revert a rejection of the kidney graft.

This case was complicated with *Listeria* sepsis with hemolysis and thrombocytopenia. The latter seems to be due mainly to the depressive action of azathioprine on the bone marrow. Consumption coagulopathy which, together with hemolysis, has been reported in *Listeria* sepsis (10) cannot have been present to a significant extent, as the prothrombin time did not increase.

The *L. monocytogenes* grown were fully sensitive to ampicillin which is reported to be the drug of choice (4). In spite of the i.v. administration of this drug in high doses the spinal fluid after 3 days of treatment still contained viable *L. monocytogenes*. Similarly in a 25-year-old kidney-transplanted woman, in New York, with *Listeria* meningitis treated with i.v. ampicillin the spinal fluid still grew *L. monocytogenes* after 3 days of treatment. However after 3 weeks of treatment with ampicillin the patient was cured and the spinal fluid had normalized (7).

New *in vitro* investigations have shown that penicillin or ampicillin in combination with kanamycin or gentamicin kill *L. monocytogenes* earlier and more effectively than any of the agents alone (3).

The antibiotic treatment given in case 2 seems, however to have been effective against *L. monocytogenes* as this microorganism was not demonstrated at autopsy. The characteristic feature of this case was the massive hemorrhages due to the pronounced thrombocytopenia. The meningeal changes were modest and the tissue reaction sparse.

The sudden decrease in kidney function appeared during high fever when the meningeal symptoms evolved and the BP fell. The myocardial infarction must have developed at about this time when the hemolysis was also demonstrated. These factors seem to have been responsible for the acute renal

failure whereas acute rejection does not seem to have been present.

From experiments with mice it is known that corticosteroids given at the beginning of the infection with *L. monocytogenes* in a dose corresponding to that given in case 2, greatly suppress the production of immunologically committed lymphocytes by depletion of lymphocytes in lymphoid tissue and by inhibition of DNA synthesis in lymphocytes (8). This leads to diminished proliferation and accumulation of macrophages at infective foci in the tissues (8).

At the same time there is an enhanced accumulation at the infective foci of polymorphonuclear leucocytes, which cannot neutralize *L. monocytogenes*. This probably represents an ineffective attempt by the host to compensate for the deficit of macrophages at the infective foci (8).

In this context it is emphasized that the first spinal fluid of case 2, who received a massive steroid dose revealed polynuclear pleiocytosis. In case 1 who received a comparatively small steroid dose, the spinal fluid was mononuclear pleiocytotic. So it is possible that the pleiocytosis other things being equal becomes increasingly polynuclear with increasing steroid immunosuppression.

CONCLUSION

The necessary immunosuppression after renal allotransplantation implies an increased risk of clinical listeriosis. One must therefore be prepared to meet cases of *Listeria meningitis* among kidney transplanted persons specially when the immunosuppression has just been increased. The diagnosis should be made rapidly and as soon as cerebrospinal fluid and peripheral blood have been obtained for culture antibiotic treatment should be started at once. In view of the seriousness of the infection, it seems justified to increase the resistance of the patient by temporarily discontinuing azathioprine and other cytotoxic drugs and reducing the steroid dose at the height of the infection. This does not necessarily involve rejection of the transplant. The kidney function should however be observed carefully.

ADDENDUM

Since the preparation of this manuscript we have had two more cases of listeria meningitis among kidney transplanted patients. They appeared shortly after 3 and 11 days, respectively intensified immunosuppression with methylprednisolone in high doses (11 g and 3 g given over 2 and 3 days, respectively) carried out to revert rejection of the kidney grafts. In both cases polynuclear pleiocytosis was demonstrated in the spinal fluid. Azathioprine was discontinued temporarily and prednisone was reduced in both patients who recovered after treatment with ampicillin plus gentamicin.

REFERENCES

1. Bogen-Møller J Hansen Listeriosis. Acta path microbiol scand. sect. B Suppl. 229 1972.
2. Bucher L-H & Schneider S S Clinical and laboratory aspects of *Listeria monocytogenes* infections. Amer J Med. 45:904 1968.
3. Gordon R, El Barrett F F & Clark D J Influence of several antibiotics singly and in combination, on the growth of *Listeria monocytogenes*. J. Pediatr 80: 667 1972.
4. Lavetter A., Leedom J M, Mathies A W, Ivier D & Wehbe P F Meningitis due to *Listeria monocytogenes*. New Engl J Med. 285 598, 1971.
5. Lourda D B, Hensle T, Armstrong D, Collins H, S Blevins A, Knappson D & Buse M Listeriosis complicating malignant disease. Ann. intern Med. 67 261 1967.
6. Mocetti T, Albert H, Wegmann W & Scherlis W Listeriosis nach Nierentransplantation. Schweiz. Med. Wochr 99 1147 1969.
7. Niraul G, Glaberman S, Harnov M, Lester E & Barrows L. *Listeria monocytogenes* meningitis during immunosuppression. New Engl J Med 285 1323 1971.
8. North R. J The action of cortisone acetate on cell-mediated immunity to infection. J exp Med. 134 1483 1971.
9. — The action of cortisone acetate on cell-mediated immunity to infection: Histogenesis of the lymphoid cell response and selective elimination of committed lymphocytes. Cell Immunol. 3 501 1972.
10. Plant M & Gardner P *Listeria monocytogenes* sepsis with disseminated intravascular coagulation. Stn. med. J (Bigham Ala.) 65, 490 1972.
11. Sampson J F *Listeria monocytogenes* meningitis: an opportunistic infection. J Neurol. Neurosurg. Psychiatr. 34 657 1971.
12. Sampson J F, Ladd J P & Hare J D Listeriosis complicating lymphoma. Amer J Med 43 39 1967.
13. Touraine J L, Revillard J P & Traeger J Biologie de l'infection listérienne. Influence de l'immunosuppression. Nouv. Presse méd. 1 2627 1972.
14. Touraine J L, Toussaint C, Blanc M & Traeger J Listeriose après transplantation rénale. Nouv. Presse méd. 1 2813 1972.

Congress Announcements

Ninth Miles International Symposium—cell membrane receptors for viruses antigens and antibodies polypeptide hormones and small molecules—will be held at the Johns Hopkins Medical Institutions, June 4-6 1975

Further information E G Bassett Ph D
Miles Laboratories Inc Elkhart, IN 46514 USA

Les prochaines Journées Internationales de Cardologie de Paris auront lieu à la Faculté de Médecine Pitié Salpêtrière sous la direction des Professeurs J Facquet et J J Weill les 12, 13 et 14 Mai 1975

Renseignements et inscriptions Professeur
J J Weill Hôpital Fernand-Widal 200 fg Saint Denis F 75010 Paris France

XXIV^e Congrès de l'Association des Pédiatres
Paris France 8 9 et 10 Juillet 1975 Nouvelle
Faculté de Médecine 45 rue des Saints-Pères,
F 75006 Paris *Secrétaires généraux* Professeurs
A A Hennequet et B Leveque *Secrétariat du*
Congrès Expansion Scientifique Française 15
rue Saint-Benoît F 75278 Paris Cedex 06 France.

RESECTION OF LEFT VENTRICULAR ANEURYSM— LATE RESULTS

I. Cullhed, W. Delius, L. Björk, A. Hallén and L. Nordgren

From the Departments of Cardiology, Diagnostic Radiology, Thoracic and Cardiovascular Surgery and Clinical Physiology, University Hospital, Uppsala, Sweden

Abstract. A survey of 22 patients operated on with left ventricular (LV) infarctectomy during 1967-72 is given. Clinical, haemodynamic and angiographic results are discussed. In most patients, in whom pre- and postoperative examination was possible, there was improvement concerning anginal pain, dyspnoea and attacks of ventricular tachycardia. Exercise studies revealed a lower heart rate at follow-up. In general, heart size had decreased. Angiographically there was decrease in end-diastolic and end-systolic heart volumes postoperatively with an increased LV ejection fraction.

In some patients with ischemic heart disease (IHD) left ventricular (LV) function can be improved by surgical intervention aimed at either increasing the oxygen supply such as revascularization procedures or decreasing the oxygen demand by reducing the LV diameter. The latter approach involves the resection of a postinfarction scar. This scar may or may not form a bulge or aneurysm. In both cases it may have a similar effect on the function of the LV. Although the term infarctectomy would be preferable, the procedure is traditionally called aneurysmectomy. The most common indications for this operation are LV failure and angina pectoris, other indications being thromboembolism and ventricular arrhythmias resistant to drug treatment.

The aim of this paper is to report the long-term clinical, haemodynamic and angiographic results of aneurysmectomy in a group of patients in whom the operation was performed electively. In only one case was the operation combined with a saphenous vein coronary artery bypass.

Present address: 1. Medizinische Klinik rechts der Isar der Technischen Universität München D-8000 München 80, West Germany.

MATERIAL

A total of 31 patients have been operated on between 1960 and April 1972. Nine patients were operated on in 1960-66, with a hospital mortality of 5 and a late mortality of 3, leaving one survivor. Part of this group has been reported earlier (5). Twenty-two patients were operated on after 1967 with a hospital mortality of 4 (18%), late mortality of 2, and 16 surviving. Two patients, still alive 11 and 3 months after operation, are not included since they are being followed up in other hospitals.

Some clinical data on the remaining 14 patients are presented in Table 1. Only one of the patients was female. The mean age was 51.4 years (range 34-65). All patients had suffered at least one myocardial infarction (MI) 2-30 months preoperatively. The limiting subjective symptom was angina pectoris and/or effort dyspnoea in 11 cases, ventricular arrhythmias in 2 and general tiredness in one case (no. 9). The patients were classified according to the NYHA classification with modifications (24). All cases but 2 were severely incapacitated.

METHODS

Pre- and postoperative evaluation. This included a 12-lead ECG and, when the condition of the patient allowed, a standardized bicycle exercise test in sitting position. Heart rate (HR) and indirect arterial pressure were noted during and at the end of the test, as well as the limiting factor, e.g. subjective symptoms, or high HR. In patients of this type we seldom allow the HR to exceed 130 beats/min.

The heart volume (HV) in sitting posture was determined radiologically (19). Angiocardiography was performed either by injecting into the left atrium (LA) through a transseptally introduced catheter or directly into the left ventricle (LV) through a grey Ödman-Löfdén catheter introduced retrogradely via the femoral artery by percutaneous technique. Contrast medium 0.3-0.5 ml/kg (Iopapag[®] 350 Nygaard, Oslo) containing 350 mg iodine/ml was injected at a rate of 16 ml/sec. Simultaneous biplane cine recording of the LV was obtained in right and left anterior oblique projections. Nine-inch images in-

Table I Patient material

B=before A=after operation

Case no	Age at op. (y)	Sex	Months from MI to op	Date of infarctectomy	Follow-up period (mo.)	Symptoms		Functional NYHA classification	
						B	A	B	A
1	57	♂	6	Dec 1967	58	Angina	Dyspnoea	IIIb	IIIa
2	57	♂	5	Jan 1968	57	Angina, dyspnoea	Angina	IIIb	II-IIa
3	60	♂	2	May 1968	52	Angina, dyspnoea	Angina	IV	IIIa
4	49	♂	9	Jan. 1969	42	Serious dysrhythmias		IIIb	II
5	61	♂	15	May 1969	39	Dyspnoea, angina		IIIb	II
6	51	♀	13	Oct 1969	35	Dyspnoea, angina		IV	II
7	65	♂	11	Dec 1969	33	Dyspnoea		IIIb	II
8	34	♂	13	Oct. 1970	23	Angina	Angina	IIIb	IIIa
9	46	♂	8	May 1971	16			II	II
10	55	♂	5	June 1971	15	Dyspnoea, angina	Dyspnoea	IIIb	II
11	45	♂	29	Oct. 1971	11	Dyspnoea	Dyspnoea	IV	IV
12	54	♂	12	Nov 1971	9	Dyspnoea	Angina	IIIb	IIIa
13	54	♂	1962, 1971	March 1972	6	Serious dysrhythmias		IV	II
14	59	♂	18	March 1972	6	Angina		IIIa	II

transflectors and 35 mm cameras (Arriflex) running at 50 or 75 frames/sec were used. As a rule only one injection was administered to each patient. However in a few cases a second injection was given after changing the patient's posture slightly to show the size of the aneurysm more clearly. The cineangiograms were analysed in an optical viewer and the contours of the LV were traced in right anterior oblique projection at end-systole and end-diastole. In addition, the size of the aneurysm was measured and expressed in per cent of the total circumference of the LV in diastole. The volume of the LV was determined using the method described previously (3). Coronary arteriography was not performed routinely preoperatively and in no case at follow-up.

Haemodynamic investigations were carried out in 12 cases. In the other 2 cases only a preoperative LV pressure measurement was performed. More complete pre- and postoperative examination was possible in only 6 cases at rest, and in 3 during exercise. In 10 cases the postoperative evaluation comprised observations both at rest and during exercise. Cardiac output (Q) was determined preoperatively using the Fick technique and postoperatively with dye dilution technique (Cardio-Green®). The mean difference (\pm SEM) between Q values according to Fick and dye dilution in this laboratory has been shown to be 0.11 ± 0.08 l/min, when obtained simultaneously (11). All haemodynamic observations were made with the patient in the supine position.

Surgical technique The operative procedure was usually performed through a median sternotomy. In a few cases, however, a transverse bilateral thoracotomy was

performed. Total cardiopulmonary bypass was established using a Kory-Cross disc oxygenator up till 1970. After 1970 an AGA disc oxygenator was used. Moderate hypothermia was induced. In most cases the aneurysm was adherent to the pericardium. During bypass the heart was dissected free as carefully as possible in order to prevent embolization. Furthermore to prevent this risk the aorta was cross-clamped immediately before the aneurysm was incised. Most of the aneurysm, including the thrombotic mass usually attached to the wall was removed. A small rim was left in order to facilitate closure. A row of interrupted heavy silk sutures reinforced by two teflon strips was used for closure followed by a continuous row of silk. The LV was vented during re-warming. When necessary the heart was defibrillated. The heart was de-aerated and the thoracic incision closed leaving chest tubes for drainage for the first 24-48 hours postoperatively.

In case 11 the preoperative examination had revealed small mitral insufficiency. At operation the papillary muscles, the chordae tendineae and the leaflets seemed to be quite normal. The mitral insufficiency was deemed to be a relative one caused by the dilated LV and on this account the mitral valve was left intact. Preoperative coronary angiography now performed routinely in these patients, had shown total occlusion of the right coronary artery in patient 14 and saphenous vein bypass was added to the aneurysmectomy. Using the method described by Favell (13) the saphenous vein was sewed end-to-side to the ascending aorta and to the right coronary artery respectively.

Table 11 Electrocardiogram and exercise test

Case no.	ECG at rest			Exercise test			Min	HR	BP	Interrupted due to	Symptoms	Arrhythmias
	Absor med	ST depression	Inverted T	Exaggeration of Q	Disappearance of Q	W (mm)						
1	B	+			+	400	4	130	160	Cardiomegaly	Angina pectoris	
2	A	+	+	-	-	Not perf	6	108	228/180	Angina pectoris, dyspnoea	Angina pectoris, dyspnoea	+
3	B		-	+	+	300	4	134	220/130	ECG reaction abnormal	-	+
4	A	+	+	-	+	450	2	138	170/95	Angina pectoris	Angina pectoris	+
5	A	+	+	-	+	600	6	132	170/95	Cardiomegaly + HR	-	-
6	A	+	+	-	+	400	6	90	165/95	Arrhythmias	-	+
7	B	+	+	+	+	Not perf	6	131	185/100	Cardiomegaly + HR	Dyspnoea	-
8	A	+	+	+	+	300	3	94	180/90	Fatigue	Fatigue	
9	B	+	+	+	+	100	4	120	115/90	Dyspnoea	Dyspnoea, fatigue	+
10	A	+	+	+	+	400	6	130	180/95	Cardiomegaly + HR	-	
11	B	+	+	+	+	600	3	128	190/100	Dyspnoea, fatigue	Dyspnoea, fatigue	-
12	A	+	+	+	+	400	6	146	185/90	Angina pectoris, dyspnoea, fatigue	Angina pectoris, dyspnoea, fatigue	-
13	B	+	+	+	+	1 600	6	150	180/90	Cardiomegaly HR	Dyspnoea	-
14	A	+	+	+	+	700	3	144	150/80	Dyspnoea	Dyspnoea	-
15	B	+	+	+	+	400	5	164	145/80	Dyspnoea + VCS	Dyspnoea	-
16	A	+	+	+	+	Not perf	6	119	150/100	Cardiomegaly	Fatigue	+
17	B	+	+	+	+	100	3	128	170/100	Fatigue + angina pectoris	Fatigue, angina pectoris	-
18	A	+	+	+	+	Not perf	6	132	180/90	Angina pectoris, cardiomegaly	Angina pectoris, fatigue	-
19	B	+	+	+	+	300	6	123	175/95	Angina pectoris + III	Angina pectoris	-
20	A	+	+	+	+	600	6	136	180/90	Cardiomegaly HR	Fatigue	-

↓ less abnormal

Table III Haemodynamic findings

B=before A=after operation

Case no	Rest															
	HR (beats/min)		Q (l/min)		CI (l/min m ²)		SV (ml)		PA (mmHg)		PCV/LA (mmHg)		LV			
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
1	67	92	5.1	9.2	2.5	4.8	76	99	21/9							
2	99		5.5		2.7		55		41/19							700/1
3		112		6.2		3.1		55				6		220/7		
4		60		5.5		2.4		92				5		100/6		120/1
5	80	64	4.9	3.8	2.9	2.2	61	99	45/23		20*	0	136/23			
6	80												135/10			
7	95	75		2.9		1.7		38				1		120/25		112/5
8	70												105/4			
9	73	79	9.3	8.7	4.3	4.1	127	110	27/11		6	6				
10	84	62	4.9	4.4	2.7	2.4	58	70	32/16		22*	3	105/19		110/8	
11	104	116	3.3	3.7	1.6	1.8	32	32	58/36		46/18	34	28	110/43	92/74	
12	118	93	2.8	4.3	1.6	2.5	23	47	74/57		29*	11		128/1		
13	90	54		4.5		2.8		83				7		86/14	120/9	
14	71	95	4.6	4.7	2.5	2.5	65	49	13/6		5	3		120/5		

Measured as PCV pressure

RESULTS

Subjective symptoms (Table I). Twelve patients improved and two were unchanged at follow-up. One of the latter (no. 11) was in advanced heart failure at operation and died 11 months after operation. Of 12 patients who were in function class IIIb-IV before operation, 11 improved and 1 became more or less symptom-free. Patient 9 had mild and moderately severe symptoms before operation, probably due to a hyperkinetic state (Table III).

Eight patients complained of angina pectoris before operation and this symptom disappeared in four. Also 8 cases had dyspnoea before operation and this persisted in only 4. Two cases were severely incapacitated preoperatively by ventricular tachycardias; at follow-up they were symptom-free and one patient had resumed his earlier physically demanding work. In this series the subjective results showed no correlation with the patient's age nor with the length of follow-up. Of 11 patients below the age of 60, only one returned to work.

Electrocardiogram (Table II). In 4 cases some degree of intraventricular conduction disturbance appeared postoperatively. A pathological Q wave was present preoperatively in 12 cases; it was unchanged at reinvestigation in 5 and showed regression in 7 cases. A pathological ST elevation in 11 cases

became lower or normal in 8 cases. The T waves were as a rule unchanged. An aneurysmal ECG pattern, i.e. a deep and broad Q or QS wave followed by ST elevation, was present in 8 cases (57%) preoperatively and in 4 at follow-up.

Exercise studies (Table II). A postoperative work test was carried out in 12 cases. The initial work load was chosen according to the individual's expected capacity. The maximal work load (W_{max}) varied between 200 and 700 kpm/min (30-110 W) and 8 patients performed at this load for 6 min. A comparison with the preoperative exercise test was possible in 8 cases and showed no difference in maximal work performed. However when the same submaximal loads were compared, there was a significantly lower HR ($p < 0.001$) at follow-up. Similar subjective symptoms appeared during the tests before and after operation. The limiting factor was angina pectoris and/or dyspnoea in 6 cases. Arrhythmias appeared in 6 cases but were a limiting factor only in one case. In the remaining 5 patients the exercise test was discontinued because of unspecific symptoms such as general fatigue.

Haemodynamic findings (Table III). Cardiac index (CI) at rest was below 2.6 l/min m² in 4 of 8 cases before and in 7 of 11 cases after operation. A direct comparison was possible in 7 cases showing an increase of at least 20% in 2 cases and a similar

Exercise

Work load (W)		HR (beats/min)		\dot{Q} (l/min)		CI (l/min m ²)		SV (ml)		PA (mmHg)		PCV/LA (mmHg)	
B	A	B	A	B	A	B	A	B	A	B	A	B	A
25	15	91	112	8.8	12.6			97	107	34/18			
	30		132		6.7		3.4		50			54	
	40		85		10.0		4.3		113			10	
	50		104		6.4		3.8		62			6	
	50		128		6.5		3.8		51			31	
65	30	93	99	14.8	13.1	6.9	6.2	133	132			7*	9
	30		85		6.1		3.4		64			8	
	20		118		6.3				53			35	
	50		95		9.9		6.2		104			27	
40	50	118	131	8.7	10.3	4.7	5.6	73	79	26/15	15	6	

decrease in one case. In case 9 \dot{Q} and stroke volume (SV) were high both before and after operation.

\dot{Q} during supine exercise was measured in 10 cases postoperatively. With the exception of case 3 increases of 20–50% from the resting value were noted the degree being related to the work load. In the three patients, in whom a comparison was possible between pre- and postoperative \dot{Q} values during exercise variations could be explained by differences in loads.

The LV filling pressure (LVFP) at rest was measured directly or indirectly in 13 cases before and in 12 cases after operation. LVFP above 18 mmHg was found in 7 cases before and in 2 cases after operation. LVFP during exercise remained normal in 5 cases and rose in 4 cases to values between 27 and 54 mmHg. The highest value 54 mmHg during exercise was found in a 60-year-old man whose \dot{Q} was unchanged during work and at rest.

Radiologic findings (Table IV). The preoperative total HV was increased in most cases and exceeded 1500 ml in 6 cases. At follow-up most hearts were smaller with a decrease in 9 cases of 50–830 ml. In 3 cases the HV had increased 75–220 ml. The total HV showed no close correlation with the LV end-diastolic volume or with the size of the scar.

The findings at LV angiography are presented in Table IV and Fig. 1. A local akinetic area persisted

after operation, but was considerably reduced in most cases. The ejection fraction determined angiocardiographically was low in all but one case preoperatively. There was a negative correlation between the end-diastolic LV volume and the ejection fraction ($r = -0.59$ $p < 0.025$). The end-diastolic volume was correlated with LVFP ($r = 0.77$ $p < 0.005$). After operation the end-diastolic and end-systolic volumes decreased in all cases. The ejection fraction was considerably larger at follow-up. No complications were connected with the pre- and postoperative examinations.

DISCUSSION

In necropsy studies cardiac aneurysm is defined as a localized out-pouching of the cavity of a cardiac chamber with or without outward bulging of the external surface (23). The frequency of postinfarction aneurysms in necropsy series varies around 15% of cases with MI (1, 12, 23). With the advent of LV cineangiography it has been demonstrated that myocardial scars with resolving local contraction disturbances are common in cases with IHD (4, 6, 10). These local changes have been categorized as akinetic, asyncretic or hypokinetic, asynchronous and dysknetic or with systolic paradoxical expansion (18). In some series akinesis and hypokinesis are more common than dyskinesis

Table IV Radiologic findings

B=before A=after operation: EII=end-diastolic, ES=end-systolic

Case no	Total HV (ml)		LV volumes (ml)				Ejection fraction		Site of wall dysfunction	Estimated size of wall dysfunction (%)
	II	A	ED		ES		B	A		
			B	A	B	A				
1	1 180	1 000	—	—	—	—	—	—	Inferior	23
2	1 700	1 510	360	210	270	80	0.25	0.62	Inferior	42
3	930	820	260	160	200	70	0.23	0.56	Inferior	31
4	1 640	1 460	260	180	200	100	0.23	0.44	Inferior	30
5	1 700	1 080	370	140	320	50	0.13	0.64	Anterior and apex	47
6	860	810	175	—	65	—	6.63	—	Anterior	23
7	1 240	800	—	110	—	50	—	0.55	Anterior	40
8	785	860	270	—	170	—	0.37	—	Anterior	25
9	1 170	1 270	170	130	130	60	0.24	0.54	Anterior	25
10	1 780	930	230	190	150	70	0.35	0.63	Anterior	33
11	2 080	2 300	420	—	330	—	0.21	—	Anterior	38
12	1 620	1 100	330	210	280	100	0.20	0.48	Anterior	48
13	1 400	1 060	250	170	190	90	0.24	0.47	Anterior and apex	57
14	940	890	280	190	180	70	0.35	0.63	Inferior	48

(2, 4, 17) Local contraction disorders were found in 27 of 52 cases (52%) 35 of 100 cases (35%) and in 42 of 66 cases (64%) in three reported series of patients with coronary heart disease (CHD) (4, 6, 17). The ventriculograms were taken with rates of 40, 30 and 6–12 frames/sec respectively which may have influenced the results to some extent. In a series of patients who had chronic postmyocardial infarction heart failure the incidence of localized contraction disturbance was 38 of 50 patients (76%) (2).

Thus local contraction disorders are common in chronic IHD. The consequences for L.V. function depend on the size of the non-contractile area (18, 20). When L.V. function is severely impaired left heart failure will appear. This failure can be clinically manifest at rest or only during exercise or can be latent without subjective symptoms but with haemodynamic or angiographic manifestations at rest or during exercise.

There are few data available concerning the incidence of different degrees of L.V. failure in patients after L.V. aneurysm resection. In the excellent review by Graber et al. (15) 16 of 18 surviving cases had a subjective and objective improvement according to clinical findings, chest X-ray and work tolerance. In only one of these cases could the results be demonstrated by means of pre- and post-operative haemodynamic examinations. In other

reports too only a few haemodynamic and angiographic reinvestigations have been described (7). A pre- and postoperative angiographic analysis of L.V. function was published recently (20) as was a haemodynamic study (16).

In recent years aneurysmectomy is often combined with direct revascularization. However even in the future isolated aneurysmectomy will be carried out in cases with diffuse or severe coronary artery disease. For this reason the long-term results of aneurysmectomy with haemodynamic and angiographic studies are of interest.

The prognosis of L.V. postinfarction aneurysm has been reported to vary from a 5-year survival figure of 12% (23) to 27% (9) and 69% (1). The cause of death is usually L.V. failure or reinfarction. This illustrates that as a rule CHD is progressive. Consequently the evaluation of the late results after aneurysmectomy should be limited to cases without reinfarction during the observation period and should be interpreted with reference to L.V. function.

Subjective results are difficult to evaluate and classify as they depend on many other factors besides the possible change in L.V. function e.g. coronary artery disease and extracardiac factors such as the patient's ambition and anxiety and attitudes to and possibilities for rehabilitation. In our group 11 of 14 patients claimed to be improved. Six denied



Fig 1 Tracings from cineangiograms of the left ventricle in right anterior oblique projection in 9 patients with LV aneurysm before operation (left) and at follow-up (right). The thick line shows the LV in end-diastole and the thin line is end-systole.

angina or dyspnoea as a limiting symptom in three of them these symptoms were provoked during the exercise test.

Aneurysmectomy has been reported to abolish ventricular tachycardia (8, 21, 22). In the present material two cases were operated on because of repeated ventricular arrhythmias. Postoperatively they were subjectively free from palpitations and the improvement was corroborated by findings at clinical, haemodynamic and angiographic investigations. However in both cases ventricular premature beats appeared during exercise test. Aneurysmectomy has been documented to be of value in isolated cases with severe ventricular arrhythmias but there are no controlled series and

the quantitative effect should be studied by long-term ECG recording.

Haemodynamic observations were made in 11 cases postoperatively. Although 10 cases were improved subjectively the \dot{Q} was low in the majority of cases as found by others (14). However in only a few cases was \dot{Q} determined both before and after operation. According to the postoperative angiographic LV studies the \dot{Q} could have been expected to be higher as practically every case displayed a decrease in end-diastolic and end-systolic volume and an increase in SV and ejection fraction. This discrepancy may be explained by earlier findings that SV according to angiocardigraphy is larger than with dye dilution (11). This is due to difficulties in outlining the LV inner contour.

Preoperatively the LV end-diastolic pressure was correlated with the end-diastolic volume. In those cases in whom a comparison was possible this pressure had decreased. Postoperatively the end-diastolic pressure was normal in all cases but one and there was no correlation to end-diastolic volume. It has been shown that these parameters have an only limited value in predicting the surgical result (14). Striking correlations between ejection fraction and other parameters of LV function such as end-diastolic volume and stroke work, have been found as well as with the size of the non-contractile area (17, 20). The LV function curve was shifted postoperatively towards a normal position (16).

A standardized exercise test is important in the evaluation of the surgical result. In this type of patient the exercise test is not intended to determine the maximally tolerated work load which would be potentially dangerous but the load inducing subjective symptoms or objective signs. In the absence of such changes the test was discontinued because of the HR attained or cautiousness. Four patients who before operation could not perform an exercise test, tolerated at follow-up a mean load of 65 W. Five other cases showed an improvement in the maximally attained work load with lower HR during the test. Only in two cases could the exercise test not be carried out.

REFERENCES

- 1 Abrams D L, Edelstam A, Lora M H & Miller A J. Ventricular aneurysms. *Circulation* 27: 164 (1963).
- 2 Baxley W A, Jones W B & Dodge H T. Left ventricular anatomical and functional abnormalities in

- chronic postinfarction heart failure. *Ann Intern Med.* 74: 499 1971
- 3 Björk, L. Semi-automatic construction and computer analysis of volume curves and pressure-volume curves in left ventricular cineangiography. *Acta radiol* diagnosis 10: 413 1970
 - 4 Björk, L., Cullhed, I. & Hallén, A.. Cineangiographic studies of the left ventricle in patients with angina pectoris. *Circulation* 36: 868 1967
 - 5 Björk, V. Left Ventricular aneurysm. *Thorax* 19: 162, 1964.
 - 6 Cheng, T. O. Incidence of ventricular aneurysm in coronary artery disease. *Amer J Med* 50: 340, 1971
 - 7 Cooley D. A., Hallman, G. L. & Healy W. S. Left ventricular aneurysm due to myocardial infarction. Experience with 37 patients undergoing aneurysmectomy. *Arch. Surg.* 88, 114 1964
 - 8 Couch, A.. Cardiac aneurysm with ventricular tachycardia and subsequent excision of aneurysm. *Circulation* 20: 251 1959
 - 9 Dubow M. H., Burchett, H. B. & Titus, J. L.. Post infarction ventricular aneurysm. A clinicomorphologic and electrocardiographic study of 80 cases. *Amer Heart J* 70: 743 1965
 - 10 Elliott, W. C. & Gorlin R. Coronary circulation, myocardial ischemia and angina pectoris. *Mod. Concepts Dis* 35 111 1966.
 - 11 Engloff, E. Aortic incompetence. *Acta med. scand* Suppl. 538 1972.
 - 12 Erhardt, L. R.. Clinical and pathological observations in different types of acute myocardial infarction. *Acta med. scand.* Suppl. 560 1974
 - 13 Favaloro, R. G.. Saphenous vein graft in the surgical treatment of coronary artery disease. *J thorac cardiovasc Surg* 58, 178 1969
 - 14 Fisher V. J., Alvarez, A. J., Shah, A., Dolgin, M. & Tice, D. A. Left ventricular scars. *Brit. Heart J* 36, 132, 1974
 - 15 Graber J. D., Oakley C. M., Pickering B. N., Goodwin, J. F., Raphael M. J. & Steiner R. E.. Ventricular aneurysm. An appraisal of diagnosis and surgical treatment. *Brit. Heart J* 34: 830, 1972.
 - 16 Halter J., Moccetti, T., Gattiker K. & Lichten P. Left ventricular dynamics before and after aneurysmectomy. In: *Coronary heart disease* (ed. M. Kaltenbach, H. Lichten & G. C. Friesinger), p. 228. Thieme Verlag, Stuttgart 1973
 - 17 Hamilton, G. W., Murray J. A. & Kennedy J. W. Quantitative angiocardiography in ischemic heart disease. *Circulation* 45: 1065 1972.
 - 18 Herman M. V., Heinle, W. A., Klein, M. B. & Gorlin R.. Localized disorders in myocardial contraction. *New Engl J Med* 277: 222, 1967
 - 19 Jonnell S. A method for the determination of the heart size by teleroentgenography. *Acta radiol* 20: 325 1939
 - 20 Kitamura, S., Echevarria, M., Kay J. H., Krohn B. U., Redington, J. V., Mendez, A., Zubiate, P. & Dunne, E. F.. Left ventricular performance before and after removal of the noncontractile area of the left ventricle and revascularization of the myocardium. *Circulation* 45: 1005 1972.
 - 21 Maloy W. C., Armat, J. E., Sowell B. F., Hendrix, G. H. Left ventricular aneurysm of uncertain etiology with recurrent ventricular arrhythmias. *New Engl J Med.* 285: 662 1971
 - 22 Poggi, L., Dor V., Noirelet, M., Kreitzmann P., Chauvial, G., Djane, P., Bory M. & Serradinalpi A. Traitement chirurgical des infarctus du myocarde pour troubles graves du rythme ventriculaire. *Presse méd.* 78: 2429 1970
 - 23 Schlichter J., Heffernstein H. K. & Katz, L. N. Aneurysm of the heart. *Medicine* 33: 43 1954
 - 24 Wade O. L. & Bishop, J. M. Cardiac output and regional blood flow. p. 112, Blackwell Oxford 1962.

IDIOPATHIC HYPERTROPHIC SUBAORTIC STENOSIS

Jørgen Fischer Hansen, Ole Pedersen-Bjergaard, Poul Stage and Fritz Efsen

From Medical Department B and Radiologic Department X Rigshospitalet
University Hospital Copenhagen Denmark

Abstract. The clinical and laboratory findings in 29 patients with idiopathic hypertrophic subaortic stenosis are presented. Dyspnoea during exercise, angina pectoris, syncope combined with left ventricular hypertrophy on ECG and chest X-ray and systolic ejection murmur at the apex and the left sternal border are the most important findings. The findings were different in patients below and above 30 years of age. Most of the patients below 30 years in function group I had normal heart volume on chest X-ray and syncope was related to exercise. All patients above 30 had symptoms, nearly all were in function groups II-IV and often complained of palpitations, had increased heart volume on chest X-ray, sign of enlarged left atrium or atrial fibrillation on ECG. Syncope was not related to exercise, but always associated with palpitation in patient above 35 years of age. Pathologic Q waves were found more often in the younger age group. The differential diagnosis is discussed in relation to fixed aortic stenosis, mitral valve disease, ventricular septal defect, coronary artery disease, and hypertrophic cardiomyopathy without outflow tract obstruction.

Idiopathic hypertrophic subaortic stenosis (IHSS) has attracted increasing attention in the recent years mainly for two reasons: firstly on account of the peculiar pathophysiology and secondly as an explanation of sudden death in apparently healthy young people (3, 12, 13).

The haemodynamic findings at rest and after administration of isotropic agents or β -adrenergic blockers have been appropriately defined (2, 6). However the diagnosis based on history, clinical findings, ECG and chest X-ray is considered to be difficult to establish (7, 17).

A correct diagnosis is important because IHSS may be treated with β -adrenergic blockers and/or operation (1, 3, 9, 21, 22, 28) and advice against heavy exercise may be important in the younger age groups. IHSS is a rare disease and can easily be overlooked. For these reasons it was decided to

make a retrospective study of simple clinical parameters as mentioned above to evaluate which findings are important for the diagnosis of IHSS.

PATIENTS AND METHODS

Among 498 patients with aortic stenosis IHSS was diagnosed in 29. They were examined in the Cardiac Laboratory, Medical Department B, Rigshospitalet, Copenhagen, and studied for the first time in the years 1952-72. In two patients the diagnosis was based on autopsy findings and in 27 on haemodynamic findings as described by Braunwald et al. (2). The diagnosis was subsequently verified at autopsy in three (26) and at operation in one of the latter 27 patients. A pressure gradient between the left ventricle and aorta at rest, varying between 10 and 165 mmHg, was found in the 27 patients by left heart catheterization. In addition to these findings 11 patients had pressure differences of 10-40 mmHg over the right ventricular outflow tract. All records were revised with respect to family history, symptoms and sign, ECG and PCG findings, chest X-ray and diagnosis on the first admission to the Department. The χ^2 -test was used for statistical evaluation.

RESULTS

Sex and age. Seven patients were females (mean age 37 years, range 9-60) and 22 were males (mean age 31 years, range 6-56). Twelve patients were below and 17 above 30 years of age.

Symptoms (Table I). Seven patients had no complaints when seen for the first time in the Department. They were admitted because of the accidental finding of an abnormal ECG or a heart murmur. When relating symptoms to age, a significantly ($p < 0.002$) higher number of patients above 30 years of age were in function groups II-IV, had dyspnoea in connection with exercise ($p < 0.07$) and complained of palpitations ($p < 0.05$). All patients above

Table 1 Function groups and symptoms in relation to age in 29 patients with IHSS

	No. of patients		
	<30 y	≥30 y	Total
NYHA function group			
I	10	3	13
II	2	11	13
III-IV	0	3	3
No symptoms	7	0	7
Syncope	2	4	6
Dizzy spells	2	4	6
Angina pectoris	1	6	7
Precordial oppression	2	7	9
Dyspnoea during exercise	2	13	15
Orthopnoea	0	2	2
Peripheral oedema	1	7	8
Palpitations	3	11	14
Total	1	17	29

30 years of age had symptoms. Two of the three patients in function group I had fainted and the third complained of heart palpitation.

Of 16 patients below 35 years of age six had attacks of syncope and/or dizzy spells appearing in all during or immediately after exercise. Of 13 patients of or above 35 years of age six had syncope and/or dizzy spells without relation to exertion, but in all cases there were heart palpitations. In four patients syncope or dizzy spells were the only symptoms. Three patients with angina pectoris were treated with nitroglycerin prior to or during the stay in the apartment. In one patient the angina vanished after lycerin, one felt increasing discomfort and in ¹ it was without effect.

Auscultation of the heart A systolic ejection murmur was heard in all patients. In 14 patients the maximum of the murmur was at the apex, in 13 at the left sternal border in the 3rd to 4th intercostal space (LSB), and in two the murmur was of the same grade at the apex and at the LSB. In nine patients the murmur was heard at the apex, the LSB, the right sternal border in the 2nd right intercostal space (RSB) and over the neck vessels, in ten at the apex, the LSB and the RSB, in eight both at the apex and the LSB, in one only at the apex, and in one only at the LSB. The murmur never exceeded grade 2 of 6 over the neck vessels and at the RSB, grade 4 at the apex and at the LSB, except in two patients: a 6-year-old boy with a grade 6 and a slender 29-year-old woman with a grade 5 systolic murmur at the LSB. The murmur could vary considerably in in-

tensity in the same patient from one day to another and even sometimes disappear completely. A low frequency diastolic murmur and/or a diastolic gallop rhythm was found in 12 patients. On some days it was described as a murmur and on some only as gallop rhythm in the same patient. A mitral snap was not reported in any of the patients, but in five patients several observers described a pronounced 1st heart sound at the apex. No diastolic murmur was reported at the LSB. A thrill was felt in seven patients at the apex, in one at the apex and LSB, and in one at the LSB only. The icetus was felt in all patients in 28 patients in the 5th intercostal space 1-2 cm lateral to the midclavicular line, in one patient in the 6th intercostal space at the midaxillary line.

The BP measured by the cuff method varied from 105 to 140 mmHg systolic and from 50 to 90 mmHg diastolic. The pulse pressure ranged from 35 to 60 mmHg, being above 50 mmHg in 20 patients. The pulse was quick in 11 patients and five had a pistol shot sound over the femoral arteries.

Phonocardiogram (Fig. 1) In 26 patients a PCG was available from the apex and/or the LSB. In 22 patients an ejection murmur was demonstrated. In four cases no murmur was present on the PCG, in these cases the systolic murmur reported at auscultation varied between grades 0 and 3 from one day to another. PCG from the apex showed in three patients a 3rd sound, in three a 4th sound, and in three both a 3rd and a 4th sound. In 10 patients in whom a diastolic murmur and/or gallop rhythm was reported at auscultation a PCG was available and in eight of

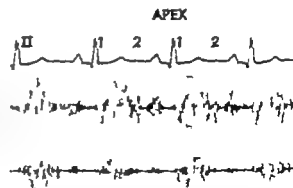


Fig. 1 PCG from the apex of a 14-year-old boy with IHSS. The ejection murmur and a pronounced diastolic murmur are noticeable. Mingograph 31 B recording 100 and 400 Hz range. ECG lead II. Paper speed 50 mm/sec.

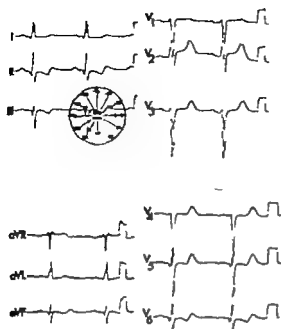


Fig 2 ECG from a 37-year-old woman with IHSS showing left ventricular hypertrophy and septal hypertrophy. Test = 1 mV.

these patients a 3rd and/or a 4th heart sound was seen on the PCG.

On the PCG from the apex of 18 patients it was possible to measure the interval from the closure of the mitral valve (M_1) to the start of the systolic murmur and from the end of the systolic murmur to the closure of the aortic valve (A_2). In 16 cases the distance from M_1 to the systolic murmur varied from 0.02 to 0.08 sec and the distance from the systolic murmur to A_2 from 0.02 to 0.08 sec. One patient, in whom the murmur started at M_1 and ended at A_2 , had a severe mitral regurgitation shown at angiocardiography and verified at autopsy. In one patient the distance was 0.02 sec from M_1 to the murmur which ended at A_2 .

The electrocardiograms were classified according to Goldman (5) modified for children according to Hansen (8). One patient had a normal ECG and two had RBBB. In 21 patients the ECG fulfilled the voltage criteria for left ventricular hypertrophy and in 10 a Q wave suggestive of ventricular septal hypertrophy was present. None of the latter patients had a history of acute myocardial infarction. In five

patients the ECG showed combined hypertrophy involving both the septum and the free wall (Fig. 2). Five patients had septal hypertrophy only (Fig. 3). Nine of 20 patients of or below 40 years of age and one of nine patients above 40 years had septal hypertrophy ($p < 0.06$).

In 23 patients the ECG showed ST segment depression and T wave inversion in V_1-V_4 . Eighteen of these patients had left ventricular hypertrophy, four septal hypertrophy as well. Four were treated with digoxin. Two of 12 patients below and seven of 15 patients above 30 years of age had broad notched P waves (>0.12 sec) in lead II as sign of left atrial hypertrophy. Two patients of 56 and 60 years of age had atrial fibrillation. Thus signs of left atrial strain were found in a significantly higher frequency in patients above than below 30 years of age ($p < 0.05$). Five of the 11 patients with ECG signs of left atrial strain had gallop rhythm at auscultation and five had dilatation of the left atrium on the chest X-ray.

Chest X-ray. The heart volume was calculated and correlated to body surface area (BSA) from chest X-rays in postero-anterior and lateral projections.

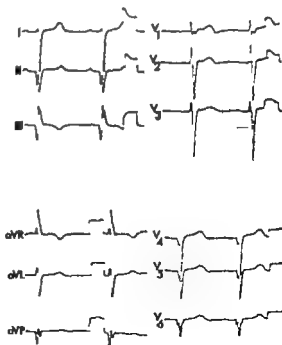


Fig 3 ECG from a 22-year-old man with IHSS showing deep Q_{V1-V3} and QS_{V1-V3} as sign of septal hypertrophy. Test = 1 mV.



Fig 4 Chest X-ray from a 22-year-old man with IHSS showing isolated hypertrophy of the left ventricle and normal lung vessels.

The configuration of the heart and great vessels was evaluated according to Meschan and Farrer Meschan (16). Two patients had normal chest X-rays. The heart volume varied considerably ranging between 310 and 720 ml/BSA (mean 540) including children. Six patients below and two above 30 years of age had normal relative heart volume (<0.05). The shape of the heart showed marked variations from normal to a diffuse enlargement involving all heart chambers.

In 18 patients bulging of the left heart border between the apex and the left atrium as a sign of left ventricular hypertrophy was seen (Fig. 4). In six patients the left ventricle was dilated. Nine patients had dilatation of the left atrium (Fig. 5) and six had a slight to moderate dilatation of the ascending aorta. Left ventricular hypertrophy and/or dilatation combined with dilatation of the left atrium was seen in seven patients, one of whom had dilatation of the aorta as well. Five patients had a combination of left ventricular hypertrophy and/or dilatation and dilatation of the ascending aorta. Five patients had signs of left heart failure. One patient showed calcification of the mitral valve.

Family history Three patients had had young siblings who died suddenly and unexpectedly and the father of one patient died in the same way. Two patients had siblings with non-obstructive

cardiomyopathy. Two of the 29 patients were siblings. In eight patients (28%) a proven or suspected family history of cardiomyopathy was thus found.

Other cardiovascular diseases In one patient an atrial septal defect and in another a ventricular septal defect was diagnosed with the platinum electrode and hydrogen. None of the patients had arterial hypertension when seen in our Department, but a 60-year-old woman had previously been treated for hypertension.

Other diseases Chronic lymphatic leukaemia, acromegaly, epilepsy and Friedreich's ataxia were diagnosed in one patient each, while two patients were treated for thyrotoxicosis.

Diagnosis on admission was in seven patients mitral stenosis and/or incompetence, in one mitral incompetence or ventricular septal defect, in four ventricular septal defect, in one Adams Stokes attacks and in one arteriosclerotic heart disease. Congenital heart disease was suggested in seven patients, and the remaining eight patients were admitted for evaluation of heart disease. None of the patients were admitted to the Department with a correct diagnosis and none on a suspicion of aortic stenosis.

DISCUSSION

The findings in our 29 patients are in agreement with other reports (2). The higher mean age of our pa-



Fig 5 Chest X-ray from 55-year-old man with IHSS showing dilatation of the left atrium, hypertrophy of the left ventricle and normal lung vessels.

tients compared with that of earlier reported IHSS patients corresponds to the increasing number of IHSS patients above 50 years of age reported by Harderson et al. (9) and Whiting et al. (27). The most important findings are a history of dyspnoea during or after exercise, attacks of syncope and angina pectoris, at auscultation a systolic ejection murmur with maximum at the apex or the LSB, on ECG left ventricular hypertrophy and on chest X-ray hypertrophy and dilatation of the left ventricle. In patients with sinus rhythm a double apex beat, pronounced neck vein pulsations and a sharp arterial pulse are prominent findings (2, 11, 15).

The physical and laboratory findings differed between the younger and older age groups (27). In the younger age group most of the patients were in function group I. Attacks of syncope were related to exercise on chest X-ray the heart volume was often within normal limits although showing left ventricular hypertrophy (15) and the ECG showed a pathologic Q wave in 50% of the cases. In the older age group all patients had symptoms and most of them were in function groups II-IV with dyspnoea, heart palpitation and attacks of syncope without relation to exercise as the prominent symptoms. Increased heart volume on chest X-ray and signs of enlarged left atrium or atrial fibrillation on the ECG.

None of our patients were admitted with a correct diagnosis. This finding accords with that of Whiting et al. (27) who reported that less than 40% of their patients were correctly diagnosed before catheterization. They found the most important differential diagnostic problems to be related to coronary artery disease (CAD), mitral regurgitation, ventricular septal defect and valvular aortic stenosis. This is in agreement with our report except for the valvular aortic stenosis. The clinical findings in fixed aortic stenosis, the systolic ejection murmur with maximum over the base and projection to the neck vessels, the weak arterial pulse, the low pulse pressure and the dilated ascending aorta on chest X-ray make this disease easily distinguishable from IHSS. Patients with combined IHSS and valvular discrete subvalvular, supra-valvular aortic stenosis or calcified aortic valves without stenosis were excluded in our series. The clinical findings in these patients are indistinguishable from pure fixed aortic stenosis and the correct diagnosis depends on haemodynamic and angiocardigraphic examinations (11, 19).

A PCG seems to be most helpful in making a correct diagnosis (10, 14). The systolic murmur is of ejection type and a delay from M to the start of the systolic murmur and/or from the end of the murmur to A₂ from 0.02 to 0.08 sec is nearly always present, as demonstrated in our patients and as reported by Lindgren and Epstein (14). This is in contrast to patients with rheumatic mitral regurgitation or other organic disease of the mitral valve in whom a true holosystolic murmur is always found at the apex. If a holosystolic murmur is heard at the apex in a patient with IHSS, an organic lesion of the mitral valve should be expected as was the case in one of Lindgren and Epstein's patients and as we have seen in one of our patients at angiocardiology and autopsy.

The findings of enlarged left atrium on chest X-ray combined with a diastolic and a faint systolic murmur (1, 3) and accentuated 1st heart sound (10) at the apex, calcification of the mitral valve and atrial fibrillation as seen in one of our patients and recently reported by Shabetal and Davidson (24) may completely simulate mitral valve disease. Lack of mitral opening snap and demonstration of an ejection murmur at the apex on the PCG should arouse a suspicion of IHSS. In these cases ultrasound echocardiography of the anterior mitral valve and the ventricular septum may give a correct diagnosis (24, 25).

Angina pectoris, a pathologic Q wave, diffusely enlarged heart or prominent left heart border on chest X-ray combined with a systolic murmur at the apex might give a diagnosis of CAD complicated by previous myocardial infarction and mitral regurgitation (4, 26). IHSS and CAD may coexist (7, 13, 17) as was the case in one of our patients with paradox effect of nitroglycerin who had a complete obstruction of the right coronary artery revealed at coronary arteriography. Signs of left ventricular hypertrophy on ECG of an ejection murmur at the apex on PCG, of a sharp arterial pulse and in some patients of paradox effect of nitroglycerin, should lead to a correct diagnosis. The final diagnosis might depend on both haemodynamic investigation and selective coronary arteriography.

A ventricular septal defect might be suspected because of medium to low frequency systolic murmur with maximum at the LSB. Demonstration of an ejection murmur on the PCG, normal flow pattern on chest X-ray, signs of left ventricular

hypertrophy which is only seen in ventricular septal defect with large shunts and increased flow pattern on chest X-ray (23) should lead to a correct diagnosis.

Hypertrophic cardiomyopathy without outflow tract obstruction may clinically be indistinguishable from IHSS and the diagnosis can only be established after haemodynamic and angiocardigraphic examinations (4, 6, 18).

REFERENCES

- Adelman A D, Wigle E D, Rangasathan M, Webb G D, Kidd B S, Bigelow W G & Silver D. The clinical cause in muscular subaortic stenosis. *Ann intern. Med.* 77 515 1972.
- Braunwald, E, Lambrew C T, Rockoff S D, Ross, J & Morrow A D. Idiopathic hypertrophic subaortic stenosis. *Circulation*, Suppl. IV 1 1964.
- Frank S & Braunwald E. Idiopathic hypertrophic subaortic stenosis. *Circulation* 37 759 1968.
- Gan G T, Goodwin, J F, Oakley C M, Oleven, E, G J, Rahimtoola, S H, Raphael M J & Steiner R. E. Q waves and coronary arteriography in cardiomyopathy. *Brit. Heart J* 34 1034 1972.
- Goldman, M J. Clinical electrocardiography 7th ed. Lange Medical Publications, Los Altos 1970.
- Goodwin J F. Hypertrophic diseases of the myocardium. *Progr. Cardiovasc. Dis.* 16 199 1973.
- Oelofte, J S, Hamby I R, Aronson, A L & Ewing, K. Coexistent idiopathic hypertrophic subaortic stenosis and coronary arterial disease. *Circulation* 46 890 1972.
- Hansen P F. Aortic stenosis. Thesis Munksgaard Copenhagen 1967.
- Hardarson, T, de la Cazaña, C S, Cuneil, R. & Goodwin J F. Prognosis and mortality of hypertrophic obstructive cardiomyopathy. *Lancet* 2. 1462, 1973.
- Harris A, Doanmoyer T & Leatham A. Physical signs in differential diagnosis of left ventricular obstructive cardiomyopathy. *Brit. Heart J* 31 301 1969.
- Kelly D T, Walzberg, E & Rowe R. D. Discrete subaortic stenosis. *Circulation* 46. 307 1972.
- Kochsiek, R, Larbig, D & Harnjanz, D. Die hypertrophische obstructive Kardiomyopathie. Springer Berlin 1971.
- Kossowsky W A, Mohr H, Dardasbi I & Gabor G E. Acute myocardial infarction in idiopathic hypertrophic subaortic stenosis. *Chest* 64 529 1973.
- Lindgren, K M & Epstein S E. Idiopathic hypertrophic subaortic stenosis with and without mitral regurgitation. *Brit. Heart J* 34 191 1972.
- Meerachswam, I S. Hypertrophic obstructive cardiomyopathy. Excerpta Medica Foundation, sterium 1969.
- Meschan I & Parter Meschan, R. M. F. Roentgen signs in clinical practice. Saunders, London 1966.
- Oran E, Gupta S, Yeo B, Nandi A C, Koh W, Lauterstein J, Potter R., Manoff A. & Piccone V. A. Idiopathic hypertrophic subaortic stenosis in patients with coronary artery disease—importance of recognition and principles of management. *Angiology* 24 538 1973.
- Ornitz, E & Pernow H. Primary cardiomyopathy. *Acta med. scand.* 192: 55 1972.
- Parker D P, Kaplan, M. A. & Connolly J E. Coexistent aortic valvular and functional hypertrophic subaortic stenosis. *Amer. J. Cardiol.* 24 307 1969.
- Peter R. H, Ormacy J G. & Beach T B. Subaortic stenosis simulating coronary disease. *Arch. Intern. Med.* 121 564 1968.
- Powell W J., Whiting, R. B, Dismore R. E. & Sanders, C. Symptomatic prognosis in patient with idiopathic hypertrophic subaortic stenosis. *Amer. J. Med.* 55 15 1973.
- Rookmaker W A, Nieveen, J, Krulnaga, K. & Bickman J R. Beta-adrenergic blockade in the treatment of left-sided hypertrophic obstructive cardiomyopathy. *Acta med. scand.* 189: 427 1971.
- Sandoe, E. Ventricular septal defect. Thesis, Munksgaard Copenhagen 1963.
- Shabetai R & Davidson, S. Asymmetrical hypertrophic cardiomyopathy simulating mitral stenosis. *Circulation* 45 37 1972.
- Shah P M, Gramiak, R. & Kramer D H. Ultrasound localization of left ventricular outflow obstruction in hypertrophic obstructive cardiomyopathy. *Circulation* 40: 3 1969.
- Simonsen J, Voigt J & Lyngborg, K. Hypertrophic obstructive cardiomyopathy. *Acta path. microbiol. scand. Sect. A* 81 174 1973.
- Whiting R. B, Powell J, Dismore R. E. & Sanders, C. A. Idiopathic hypertrophic subaortic stenosis in the elderly. *New Engl J Med* 283 196 1971.
- Wolsteinbome G E. W & O'Connor M (ed.). Hypertrophic obstructive cardiomyopathy. Ciba Foundation Study group 37. London 1971.

DIAZEPAM IN CARDIOVERSION

G Forsell R Nordlander O Nyquist and E. Ornljus

*From the Department of Medicine, Karolinska Institutet at Huddinge Hospital
Huddinge, Sweden*

Abstract Diazepam has been used to an increasing extent in cardioversion since avoiding general anaesthesia simplifies the procedure. The present study concerns the effect of diazepam on BP and blood gases in 13 cases of cardioversion. A moderate fall of both systolic and diastolic BP occurred. The arterial pO_2 and pCO_2 did not change significantly.

Diazepam has been used to an increasing extent in cardioversion in recent years (1, 2, 4, 5, 6, 7, 8, 9, 10) since avoiding general anaesthesia reduces the frequency of complications, especially the incidence of premature ventricular beats (4). Using diazepam also simplifies the cardioversion procedure as the skilled help of an anaesthesiologist is not mandatory. Diazepam is generally claimed to produce amnesia in nearly all cardioversion patients without causing any troublesome cardiovascular or respiratory depression (2, 4, 7, 8, 9, 10).

As we could not find any systematic report bearing this general opinion, we started the present study of blood pressure and blood gases in cardioversion with diazepam.

MATERIAL AND METHODS

The study concerned 13 patients with 13 cardioversion procedures. Six were males and six females, aged 52-74 years. Seven patients had chronic and one acute atrial fibrillation. In three patients the indication for cardioversion was acute and in one chronic atrial flutter. One of the 12 patients had valvular heart disease, one treated thyrotoxicosis, one cor pulmonale and four arterial hypertension. In five patients no disposing factor was found.

Patients with chronic atrial fibrillation or flutter had effective anticoagulant treatment for 3 weeks before cardioversion, if not contraindicated. Digoxin treatment was stopped 3 days before the procedure. Quinidine prophylaxis was started 1-2 days before the countershock

(quinidine dextrol 1.2 g/day) in all patients with chronic arrhythmia. The patients had fasted for at least 4 hours.

No premedication was given. The patients breathed air throughout the procedure. Diazepam was administered i.v. at a rate of about 5 mg/2-3 min until the patient became somnolent with slurred speech or fell asleep. The total dose required varied between 5 and 45 mg (mean 23). The synchronized DC countershocks were delivered through anterior chest and back paddles about 2 min after the last injection. The energy of the first shock was set to 100-300 W (mean 177). In 3 cases a second shock of 300 W was needed, which restored sinus rhythm in one case.

BP was recorded every second minute from just before the diazepam injection until about 10 min after the countershock. Blood for pO_2 and pCO_2 analysis was taken by puncturing the femoral artery just before the diazepam injection and immediately following the countershock, 4-6 min after the last injection.

When alert after the procedure, the patients were asked if they recalled anything unpleasant from the treatment.

RESULTS

Delivery of the countershock caused transient arousal and usually a groan or a slurred comment, but when alert 15-30 min later all patients denied any unpleasant event.

The BP range was 185-110/110-80 mmHg before the diazepam injection, and the minimum values after injection and cardioversion were 145-100/105-60 mmHg, a significant decrease of both pressures. The maximal systolic drop in each patient showed a range of 0-50 mmHg (mean 19) and the maximal diastolic change of -4.5-+5 mmHg (mean -14). There was no correlation between the diazepam dose given and the maximal BP fall.

The arterial pO_2 range was 69-107 before and 60-108 mmHg after the diazepam injection and cardioversion, a non-significant difference. The change was -5-+14 mmHg (mean -7). The arterial pCO_2 was 30-44 before and 30-47 mmHg after

the diazepam injection and cardioversion a non-significant difference. The change varied between -2 and $+3$ mmHg (mean 0).

DISCUSSION

The diazepam doses needed to produce somnolence/sleep and amnesia vary considerably in the present series between 5 and 45 mg i.v. In our experience elderly patients with congestive heart failure need the smallest doses and alcoholic patients the largest. A high requirement in alcoholic patients has also been reported by others (1-7). The upper dose limit varies in the literature between 25 and 80 mg i.v. (1-7) and was in the present series 45 mg i.v. If a reasonable dose of diazepam proves ineffective it has been suggested that i.v. morphine sulphate should be added (1).

In the present series amnesia was induced in all patients. In 3 pooled series totalling 74 patients the incidence of non-amnesia was 5% (4-7, 10). The present study confirms the general statement of many reports that diazepam in cardioversion does not cause any troublesome cardiovascular or respiratory depression (2, 4, 7, 8, 9, 10).

However the systolic BP dropped maximally 50 mmHg and the diastolic BP 45 mmHg (mean maximal fall 19 and 14 mmHg, respectively). A decline of this magnitude could theoretically be critical for the occasional patient but may also occur in general anaesthesia. A mean pressure fall of the same magnitude as in the present study occurred during induction of anaesthesia in patients premedicated for cardiac surgery whether barbiturates or diazepam (1-30 mg) were used (3). In that study the maximal BP effect was not reached until 6 min after the diazepam injection. In the present study only 2 min had elapsed when the countershock was elicited so the BP changes reported here probably represent the net effect of the injection plus the countershock. However the immediate object of

the present study was the cardioversion procedure as performed clinically.

The pO₂ changes were usually minor but the decrease in one patient without acute or chronic respiratory disease from 83 to 60 mmHg invites caution especially in patients with respiratory failure. In one series oxygen 5 l/min was administered routinely throughout the procedure (7). Equipment for manual ventilation should anyhow be available as occasional cases of transient apnoea have been reported though they did not require assisted ventilation (8).

We have now been using diazepam in all cardioversions for 2 years and the only complications noted are that the amnesia induced has been inadequate in 2 of about 100 patients.

REFERENCES

1. Glassman E. Direct current cardioversion. *Amer Heart J* 82: 128 1971.
2. Kerooban R. J. Diazepam in cardioversion. *Lancet* i 718 1966.
3. Lyons S. M. & Clarke R. S. J. Anaesthesia for cardioversion. *Brit med J* 4: 229 1971.
4. Macnister J. J., Rosenberg M. S., Carleton R. A. & Ormstrong J. S. Comparison between diazepam and sodium thiopental during DC countershock. *J.A.M.A.* 199: 168 1967.
5. Nutter D. O. & Massoudi R. A. Diazepam in cardioversion. *New Engl J Med* 273: 650 1965.
6. Remeikov L. Drug therapy before and after the electroversion of cardiac dysrhythmias. *Progr cardiovasc. Dis* 16: 531 1974.
7. Rotem C. E. Diazepam injectable as premedication for electrical cardioversion. *Canad. med Ass J* 103: 1381 1970.
8. Somers K., Gerstone R. F., Patel A. K. & D'Arbela P. M. Intravenous diazepam for direct-current cardioversion. *Brit. med J* 4: 13 1971.
9. Virgo L. N., Wyatt, G. M. & Lopez, J. F. Diazepam in cardioversion. *Canad. Anaesth. Soc. J* 18: 166 1971.
10. Winters W. L. J. M., Donough, M. T., Hafer J. & Dietz, R. Diazepam. A useful hypnotic drug for direct-current cardioversion. *J.A.M.A.* 204: 174 1968.

INVOLUTION OF POLYCYSTIC KIDNEYS DURING ACTIVE TREATMENT OF TERMINAL UREMIA

Jørn Hess Thaysen Erik Christensen, Antonio Alarcon-Zanta
and Børge Movild

*From Medical Department P Division of Nephrology and the Department of Radiology
Rigshospitalet Copenhagen Denmark*

Abstract Following institution of chronic dialysis and/or renal transplantation for terminal uremia, four consecutive patients observed for 15-48 months with their polycystic kidneys in situ invariably showed rapid relief of symptoms (pain, hematuria) followed by gradual but steady involution of the kidneys. The mechanism and the practical clinical implications are discussed.

a gradual reduction in kidney size was in evidence. The longer the period of observation the more pronounced was the observed involution of the polycystic kidneys. The significance of this finding is discussed from the pathogenetic and clinical points of view.

It is well established that polycystic kidneys usually are considerably enlarged before marked reduction in renal function is in evidence (1). Although the rate of growth of the polycystic kidneys in relation to the progression of the disease has not been studied in detail it appears likely that the increment in kidney size decreases during progressive renal insufficiency until the stage of terminal renal failure is reached.

Among many other observations of pathophysiological and clinical significance the advent of chronic hemodialysis and renal transplantation has made it possible to observe the development of polycystic kidney disease beyond the stage at which the patients previously died in terminal uremia. None of the larger materials on dialysis and transplantation in patients with polycystic kidneys in situ do, however, specify this problem (2-4, 5). While perusing the literature in preparation for the present paper we found only one publication illustrating a marked reduction in the size of the polycystic kidneys of a 37-year-old man two years after successful grafting (3).

The present paper supports and extends this observation. In four consecutive patients who had their polycystic kidneys in situ for more than 11 months after institution of active treatment of terminal uremia by dialysis and/or transplantation

PATIENT MATERIAL

In the period Jan 1965-June 1974 11 patients with terminal renal disease due to polycystic kidney disease were treated at Rigshospitalet.

Nephrectomized patients One patient was totally nephrectomized during dialysis and 4 were totally nephrectomized in connection with necrokidney transplantation. Four of the 5 patients were from our early experience in 1965-1969 when bilateral nephrectomy was carried out on liberal indications as the majority of all patients actively treated for terminal uremia.

Non-nephrectomized patients In 9 patients, the polycystic kidneys were left in situ. Five of them, 2 on chronic dialysis, 2 back on chronic dialysis after rejection of necrokidney grafts and 1 recently transplanted with necrokidney have been followed for less than 6 months on active treatment. In these cases possible changes in the size of the polycystic kidneys have not yet been studied due to the short observation period.

Four patients all transplanted with necrokidneys, have been observed for periods exceeding 15 months (range 15-48) and in these patients study of the clinical course and of changes in kidney size has been performed. Their case histories are presented below.

CASE HISTORIES

Patient 1 woman, born in 1930 (TP 62)

Family history Marked disposition for polycystic kidney disease on paternal side: sister suffering from polycystic kidneys died from subarachnoidal hemorrhage in 1973.

Course of illness In 1959 acute pyelonephritis followed by attacks of flank pain and macroscopic hematuria

at monthly to bimonthly intervals. In Aug. 1959 left-sided hemibotomy with puncture of cysts without evident relief. In Dec. 1959 surgical drainage of right-sided perineal abscess. Rising serum creatinine since 1962. In terminal renal failure in the spring of 1970 still having frequent attacks of recurrent flank pain and hematuria.

Diagnostic verification. I.v. urography in 1959. Inspection of kidneys at surgery in 1959: renal angiograms in March 1970 and May 1974.

Kidney and liver size before transplantation. Both kidneys considerably enlarged from 1959 to 1970. No liver enlargement.

Transplantation. was carried out without antecedent dialysis with a necrokidney on May 21 1970. Immediate onset of graft function.

Course of disease after transplantation. No episodes of flank pain or hematuria after transplantation. Kidney size gradually decreasing. Kidneys no longer palpable from Sept. 71. 16 months after grafting.

Present status (June 74. 49 months after transplantation). Excellent clinical condition. Revalidated to housework plus part-time work as secretary. Kidneys not palpable. No liver enlargement. Serum creatinine 0.09 mmol/l. 24-hour endogenous creatinine clearance 80–90 ml/min. BP 115/70. Urine less than 100 mg protein/24 hours, sterile on culture. Medication: azathioprine 100 mg and prednisone 2.5 mg/day.

Patient 2 man, born in 1919 (TP 124)

Family history. N. known cases of polycystic kidney disease in the family.

Course of disease. No definite urologic symptoms. In March 1971 examined by own doctor due to uremic symptoms. Transferred to Rigshospitalet with creatinine clearance of 8 ml/min.

Diagnostic verification. Renal angiograms in April 1971 and 1. Oct. 1972. Autopsy in Dec. 1972.

Kidney and liver size before dialysis and transplantation. Neither kidneys nor liver were palpable, possibly due to marked obesity.

Dialysis and transplantation. Regular dialysis in July–Nov. 71. Transplanted on Nov. 2, 1971 with necrokidney. Immediate function which remained good until July 1972 when chronic rejection was diagnosed clinically and by graft biopsy. Died in uremia with terminal septicaemia on Dec. 27 1972. Autopsy revealed few 1 cm large cysts in the liver and typical polycystic kidneys measuring 16 × 10 × 10 cm each.

Course of disease during dialysis and after transplantation. As before active treatment, no local urologic symptoms. Kidneys not palpable, liver not palpable.

Patient 3 man, born in 1921 (TP 133)

Family history. Marked disposition to polycystic kidney disease on maternal side. One older brother died from uremia at the age of 40. A twin brother suffering from polycystic kidney disease was transplanted at another hospital in 1973.

Course of disease. Between 1950 and renal transplantation in 1971 numerous episodes of flank pain and macroscopic hematuria. Rising serum creatinine since 1964. In terminal renal failure in 1971.

Diagnostic verification. I.v. urography in 1964. Renal angiograms in Oct. 1970 and March 1974.

Kidney and liver size before transplantation and dialysis. Kidneys large and readily palpable between 1964 and 1971. No liver enlargement.

Transplantation and dialysis. Transplanted without antecedent dialysis with a necrokidney on Dec. 3 1971. Acute rejection followed by graftectomy on Dec. 9 1971. On regular hemodialysis between Dec. 9 1971 and Feb. 2, 1973 when retransplanted with a necrokidney which is still functioning.

Course of disease on dialysis and after successful transplantation. Since admission to the regular hemodialysis program following failure of first graft there have been no episodes of flank pain or hematuria. Polycystic kidneys, gradually decreased in size were no longer palpable in July 1972, 7 months after start of hemodialytic treatment.

Present status (June 1974. 30 months after institution of dialysis and 16 months after successful transplantation). Clinically in good condition, works 6 hours per day in previous job as accountant. Kidneys not palpable. Liver 4–5 cm below right costal margin (It is not possible to state from the records when the liver which was not palpable before dialysis started to increase in size.) Serum creatinine 0.10–0.11 mmol/l. 24-hour endogenous creatinine clearance 60–65 ml/min. BP 140–150/90–100. Urine less than 150 mg protein/24 hours and sterile on culture. Medication: azathioprine 125 mg and prednisone 15 mg/day.

Patient 4 woman, born in 1914 (TP 135)

Family history. Not conclusive.

Course of disease. Slight uremic symptoms since 1966. M. flank pains or hematuria. In terminal uremia in June 1971.

Diagnostic verification. I.v. urography in 1966, renal angiograms in May 1971 and March 1974.

Kidney and liver size before dialysis and transplantation. Kidneys palpable and slowly growing since 1966. Liver not palpable.

Dialysis and transplantation. On regular dialysis between June 1971 and Dec. 1 1971 when transplantation with a necrokidney which is still functioning was performed.

Course of disease on dialysis and after transplantation. As before, no flank pain, no hematuria. Kidneys very large and readily palpable at transplantation in Dec. 1971. Kidneys still palpable in Jan. 1973, no longer palpable in Aug. 1973. 26 months after start of dialysis and 20 months after grafting.

Present status (June 74. 36 months after institution of dialysis and 30 months after successful transplantation). General condition good. Receives pension but manages full-time housework. Kidneys not palpable, liver not enlarged. Serum creatinine 0.09 mmol/l. 24-hour endogenous creatinine clearance 60–70 ml/min. BP 140–160/90–100. Urine less than 100 mg protein/day. Intermittent asymptomatic bacteriuria. Graft program normal. Medication: azathioprine 100 mg and prednisone 5 mg/day.

ANGIOGRAPHIC TECHNIQUE

All patients with terminal renal failure are examined with renal angiograms and arteriography of the iliac vessels as part of our "pretransplantation survey". Routine ultrasonic scanning was not carried out and i... angiography even with high doses of contrast and nephrotomography does not reveal the kidney area in polycystic kidneys with very poor function. Therefore, apart from physical examination the only possibility of re-examining the patients with the aim of demonstrating a change in kidney size was to repeat the renal angiograms.

Like urography renal angiography does not give a sharp delineation of the kidney surface in polycystic kidneys with exceedingly low function. It was therefore necessary to search for the most peripheral arterial ramifications in the right and left kidneys which should be verified as anatomically identical in the first and the second renal angiogram of each patient. The ramification points were connected by straight lines, and the area of the resulting polyeders was measured by planimetry (Fig 1). The percentage reduction in the size of the polyeders was taken to represent the percentage reduction in total kidney size.

Possible errors caused by slight variations in the film-focus distance were corrected for by planimetry of the area of the spine corpora vertebrae in both examinations. This correction only proved to be of importance in one case, in whom the first and second angiograms were carried out by slightly different techniques.

RESULTS

Clinical symptoms and signs As illustrated by the case histories hematuria and flank pain subsided rapidly following grafting (pat. 1) as well as after institution of regular dialysis (pat. 3). In the three patients in whom the kidneys were clinically palpable (nos. 1, 3 and 4) they gradually diminished in size and were no longer palpable 16 months after grafting (pat. 1), 7 months after institution of regular dialysis (pat. 3) and 20 months after grafting (pat. 4).

Thus clinical symptoms apparently vanish long

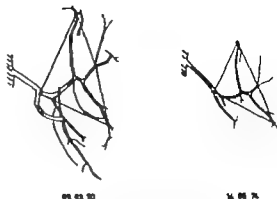


Fig. 1 The most peripheral arterial ramifications which could be shown to be identical in two arteriograms on patient 1 taken with an interval of 30 months.

before reduction in kidney size is in evidence. The results are summarized in Table I.

Kidney size As described above, an attempt was made to estimate the percentage reduction in kidney size by renal angiography. Table II shows that a reduction was found in all four patients and that it was roughly parallel to the active treatment period.

DISCUSSION

The present material of 4 consecutive patients with their polycystic kidneys *in situ* for more than 15 months after institution of chronic hemodialysis and/or renal transplantation demonstrates that the kidneys invariably undergo a gradual involution and that the degree of shrinkage is roughly proportional to the observation period. This supports and extends the only other published observation of a similar phenomenon in a male patient, who was

Table I Clinical data of the four patients

Patient no.	Sex	Born in	Treatment	Pain	Hematuria	Infection	Palpable kidney	Palpable liver
1	♀	1910	Before TP After TP	+++ 0	++ 0	+ 0	++ 0	0 0
2	♂	1919	Before dial. and TP After dial. and TP	0 0	0 0	0 0	0 0	0 0
3	♂	1921	Before dial. and TP After dial. and TP	+++ 0	+++ 0	() 0	+++ 0	0 ++
4	♀	1914	Before dial. and TP After dial. and TP	0 0	0 0	0 0	+++ 0	0 0

at monthly to bimonthly intervals. In Aug. 1959 left-sided lumbarotomy with puncture of cysts without evident relief. In Dec. 1959 surgical drainage of right-sided perirenal abscess. Rising serum creatinine since 1962. In terminal renal failure in the spring of 1970 still having frequent attacks of recurrent flank pain and hematuria.

Diagnostic verification I.v. urography in 1959. Inspection of kidneys at surgery in 1959. renal angiograms in March 1970 and May 1974.

Kidney and liver size before transplantation Both kidneys considerably enlarged from 1959 to 1970. No liver enlargement.

Transplantation was carried out without antecedent dialysis with a necrokidney on May 21 1970. Immediate onset of graft function.

Course of disease after transplantation No episodes of flank pain or hematuria after transplantation. Kidney size gradually decreasing. Kidneys no longer palpable from Sept. 71. 16 months after grafting.

Present status (June 74. 49 months after transplantation). Excellent clinical condition. Revaluated to housework plus part-time work as secretary. Kidneys not palpable. No liver enlargement. Serum creatinine 0.09 mmol/L; 24-hour endogenous creatinine clearance 80–90 ml/min. BP 115/70. Urine less than 100 mg protein/24 hours, sterile on culture. Medication: azathioprine 100 mg and prednisone 2.5 mg/day.

Patient 2 man, born in 1919 (TP 124)

Family history No known cases of polycystic kidney disease in the family.

Course of disease No definite urologic symptoms till March 1971. Examined by own doctor due to uraemic symptoms. Transferred to Rigshospitalet with creatinine clearance of 8 ml/min.

Diagnostic verification Renal angiograms in April 1971 and in Oct. 1972. Autopsy in Dec. 1972.

Kidney and liver size before dialysis and transplantation Neither kidneys nor liver were palpable, possibly to marked obesity.

Dialysis and transplantation Regular dialysis in July–Nov 71. Transplanted on Nov. 2 1971 with necrokidney. Immediate function, which remained good till July 1972, when chronic rejection was diagnosed clinically and by graft biopsy. Died in uremia with terminal septicaemia on Dec. 27 1972. Autopsy revealed few 1 cm large cysts in the liver and typical polycystic kidneys measuring 16×10×10 cm each.

Course of disease during dialysis and after transplantation As before active treatment, no local urologic symptoms. Kidneys not palpable, liver not palpable.

Patient 3 man, born in 1971 (TP 133)

Family history Marked disposition to polycystic kidney disease on maternal side. One older brother died from anemia at the age of 40. A twin brother suffering from polycystic kidney disease was transplanted at another hospital in 1973.

Course of disease Between 1950 and renal transplantation in 1971 numerous episodes of flank pain and macroscopic hematuria. Rising serum creatinine since 1964. In terminal renal failure in 1971.

Diagnostic verification I.v. urography in 1964. Renal angiograms in Oct. 1970 and March 1974.

Kidney and liver size before transplantation Kidneys large and readily palpable between 1964 and 1971. No liver enlargement.

Transplantation and dialysis Transplanted at antecedent dialysis with a necrokidney on Dec. 3, 1971. Acute rejection, followed by graftectomy on Dec. 9 1971. On regular hemodialysis between Dec. 9 1971 and Feb. 1973 when retransplanted with a necrokidney which still functioning.

Course of disease on dialysis and after successful transplantation Since admission to the regular hemodialysis program following failure of first graft have been no episodes of flank pain or hematuria. Polycystic kidneys gradually decreased in size were no longer palpable in July 1972, 7 months after start hemodialytic treatment.

Present status (June 1974. 30 months after institution dialysis and 16 months after successful transplantation). Clinically in good condition, works 6 hours per day previous job as accountant. Kidneys not palpable. 4–5 cm below right costal margin. (It is not possible state from the records when the liver which was palpable before dialysis started to increase in size. Serum creatinine 0.10–0.11 mmol/L. 24-hour endogenous creatinine clearance 60–65 ml/min, BP 140–90–100. Urine less than 130 mg protein/24 hours, sterile on culture. Medication: azathioprine 125 mg prednisone 15 mg/day.

Patient 4 woman, born in 1914 (TP 135)

Family history Not conclusive.

Course of disease Slight uraemic symptoms since 1966. No flank pains or hematuria. In terminal anemia in Jan. 1971.

Diagnostic verification I.v. urography in 1966, angiograms in May 1971 and March 1974.

Kidney and liver size before dialysis and transplantation Kidneys palpable and slowly growing since 1966. Liver not palpable.

Dialysis and transplantation On regular dialysis between June 1971 and Dec. 21 1971 when transplanted with a necrokidney which is still functioning was formed.

Course of disease on dialysis and after transplantation As before, no flank pain, no hematuria. Kidneys very large and readily palpable at transplantation in Dec. 1971. Kidneys still palpable in Jan. 1973. No longer palpable Aug. 1973. 26 months after start of dialysis and 20 months after grafting.

Present status (June 74. 36 months after institution of dialysis and 30 months after successful transplantation). General condition good. Receives pension, but manages full-time housework. Kidneys not palpable. Liver not enlarged. Serum creatinine 0.09 mmol/L. 24-hour endogenous creatinine clearance 60–70 ml/min. BP 140–160/90–100. Urine less than 100 mg protein/day. Intermittent asymptomatic bacteriuria. Graft program normal. Medication: azathioprine 100 mg and prednisone 5 mg/day.

RENAL EXCRETION OF VANCOMYCIN IN KIDNEY DISEASE

H. E. Nielsen, H. E. Hansen, B. Korsager and P. E. Skov

From the First Medical University Clinic and the Department of Medical Bacteriology
Århus Kommunehospital Århus, Denmark

Abstract The renal elimination of vancomycin has been determined in 18 patients. In 4 anuric patients in intermittent haemodialysis the dosage of vancomycin necessary to treat infections with penicillin-resistant strains of *Staphylococcus aureus* was determined. In 14 patients with varying degrees of renal (insufficiency) vancomycin, creatinine and ¹²⁵I-iothalamate clearances were measured and found to be closely correlated. After administration of the initial vancomycin dose and attainment of the serum concentration desired, the maintenance dose can be calculated on the basis of the GFR.

In patients with renal insufficiency and in kidney transplant recipients infections with *Staphylococcus aureus* and Gram-negative rods are a very common complication (5, 12). These bacteria are often resistant to commonly used antibiotics and therefore potentially ototoxic and nephrotoxic antibiotics must be employed such as streptomycin, kanamycin, gentamycin and vancomycin. Ototoxicity appears to be particularly common in patients with renal insufficiency since the elimination of these substances is primarily by way of the kidneys (3, 4, 6, 15). Treatment of renal-insufficient patients with these substances necessitates monitoring of the serum concentration.

The purpose of the present study was to investigate whether vancomycin administration could be calculated on the basis of renal function. We therefore determined the renal excretion rate for vancomycin in patients with varying degrees of renal insufficiency.

MATERIAL

Eighteen patients, 8 women and 10 men aged 1-68 years, were studied. These patients were divided into 2 groups.

Group I consisted of 4 patients, 3 women and 1 man,

aged 21-37 years. These patients were anuric and in chronic intermittent haemodialysis. Bilateral nephrectomy had been performed in all and one had a non-functioning renal graft. Body weight varied from 43 to 60 kg. Serum vancomycin was measured during treatment of infections caused by *Staphylococcus aureus* (Fig. 1).

Group II consisted of 14 patients with serum creatinine values between 0.8 and 19.3 mg/100 ml. ¹²⁵I-iothalamate clearance was 60-100 ml/min in 3 patients, 20-60 ml/min in 7 and less than 20 ml/min in 4. Other data are given in Table I.

METHODS

A cellophane membrane, 1 m² 11 µm thick, was used during dialysis which varied from 8 to 10 hours.

In patients in group II vancomycin, creatinine and ¹²⁵I-iothalamate clearance (C_{crea} , C_{Cr} , C_{io}) determinations were made and the clearance ratios $C_{\text{io}}/C_{\text{Cr}}$ and $C_{\text{io}}/C_{\text{crea}}$ were calculated. A classical clearance technique was employed (17).

¹²⁵I-iothalamate was supplied by Amersham Radiochemical Centre (England). Total dose was 10-25 µCi ¹²⁵I-iothalamate-hydrochloride. Measurement of vancomycin in serum and urine was done by an agar diffusion method (7). Dishes, 287/287 mm, were employed with Difco antibiotic medium no. 11 as basal layer and Difco antibiotic medium no. 1 as growth layer with *Staphylococcus aureus* PSTEL 19128 embedded as indicator organism. Sixty-four cylindrical holes were punched into the agar dish so that standard dilutions of vancomycin and all samples from single patient could be applied to the same dish.

Using stock solution of vancomycin-hydrochloride dissolved in phosphate buffer (pH 6.2) standard dilution of vancomycin with human serum was made with concentrations of 100, 80, 40, 20 and 10 µg/ml. Diameters of the inhibition zones produced by these dilutions were used to plot dose-response curve.

Zones of inhibition were measured for both undiluted and diluted (1/2) serum samples (dilution medium: human serum) and for urine undiluted and diluted (1/2 or diluted 1/5) dependent upon the expected concentration (dilution medium: 0.9% NaCl). Three to four zones of inhibition were read for each dilution and the

Table 1 Kidney function studies and excretion of vancomycin-hydrochloride in group II

CG=chronic glomerulonephritis, CP=chronic pyelonephritis, RAT=renal allotransplantation

Pat. no	Sex	Age (y)	Diagnosis	Proteinuria (g/24 h)	Serum albumin (g/100 ml)	Clearances (ml/min)			Clearance ratios	
						C_{Cr}	C_{Cr}	C_{urea}	C_{urea}/C_{Cr}	C_{urea}/C_{Cr}
1	♂	48	CG	3.6	2.9	2.6	3.3	2.4	0.92	0.69
2	♂	28	RAT	1.1	2.9	9.2	16	7.4	0.81	0.46
3	♀	44	CP	1.6	2.9	10	17	8.3	0.80	0.49
4	♂	62	Analgesic nephropathy	0.8	2.9	19	28	16	0.84	0.57
5	♀	43	RAT	0	2.4	30	32	28	0.95	0.54
6	♀	34	CP	0.8	2.8	30	33	20	0.67	0.38
7	♂	25	RAT	0.6	2.0	40	61	32	0.80	0.52
8	♂	50	Nephrocalcinosis	0	3.4	51	86	38	0.74	0.44
9	♀	22	RAT	0	2.4	34	68	38	0.70	0.56
10	♀	68	No kidney disease	0	2.8	56	85	42	0.75	0.49
11	♂	21	RAT	0	2.8	58	72	37	0.64	0.51
12	♂	55	No kidney disease	0	3.4	67	89	48	0.72	0.54
13	♂	28	RAT	0	3.0	72	119	48	0.67	0.40
14	♂	45	CG	13.5	1.3	103	123	101	0.98	0.82
Average		41			2.7	43	82	33	0.79	0.53
S.D.					0.6	28	37	24	0.11	0.11
S.E.M.					0.2	7.5	9.9	6.6	0.03	0.03

average zone diameter was used in plotting the curve. Each serum sample was represented by the result of 7-8 determinations. Concentration of serum vancomycin varied from 5.6 to 29 µg/ml (mean 18.4 ± 5.5).

RESULTS

Group I In four anuric patients serum concentrations of vancomycin were determined during use of the agent (Fig. 1). As shown

in the Figure the administration of 1 g vancomycin per week was sufficient to maintain serum concentration. This dose corresponds to 7% of mal dose. On the basis of the quite serum concentrations between doses it can be concluded that only small amounts of vancomycin transversed the dialysis membrane.

Group II In 14 patients (Table 1 Figs 2 and 3) with 125 Iothalamate clearances var-

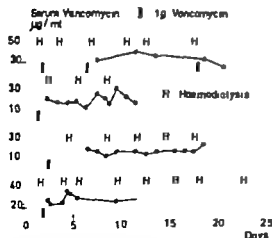


Fig. 1 Variations of serum vancomycin concentrations in patients in group I.

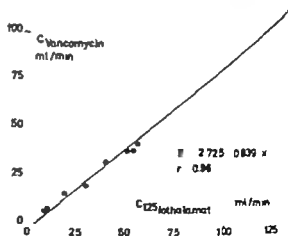


Fig. 2 Vancomycin clearance compared to 125 Iothalamate clearance in group II.

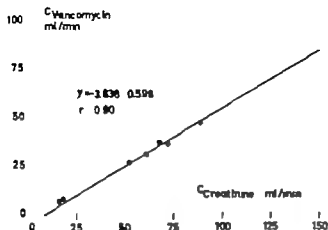


Fig. 3 Vancomycin clearance compared to creatinine clearance in group II

from 2.6 to 103 ml/min and creatinine clearances from 3.5 to 123 ml/min a good correlation was found between C_{van} and C_{cre} ($p < 0.001$ regression analysis and r -values given in Fig. 2). A good correlation was also found between C_{van} and C_{cr} ($p < 0.001$ regression analysis and r values given in Fig. 3). The $C_{\text{van}}/C_{\text{cr}}$ ratio was calculated to be 0.79 ± 0.11 and $C_{\text{van}}/C_{\text{cre}}$ ratio 0.53 ± 0.11 . Clearance ratios were independent of the degree of renal insufficiency. There was a negative limit correlation between $C_{\text{van}}/C_{\text{cr}}$ and serum albumin concentrations ($0.05 < p < 0.1$). In a patient with the nephrotic syndrome (no. 14) the $C_{\text{van}}/C_{\text{cr}}$ ratio was 0.98.

DISCUSSION

The chemical structure of vancomycin is not known but its molecular weight has been found to be approximately 1800 (13). This substance can form complexes in vitro with various peptides (13, 14). Vancomycin is an effective drug for the treatment of infections with penicillin- and methicillin-resistant strains of *Staphylococcus aureus* (1, 9, 16). Drug resistance has not been demonstrated.

Therapeutic serum concentration during vancomycin treatment of *Staphylococcus aureus* infections varied from 10 to 60 $\mu\text{g/ml}$ dependent upon the sensibility of the strain being treated. Minimum inhibiting concentration for *Staphylococcus aureus* is reported to be between 0.8 and 6.2 $\mu\text{g/ml}$ (3). Ototoxicity has been seen with concentrations between 80 and 100 $\mu\text{g/ml}$ (4). De-

velopment of renal insufficiency during vancomycin therapy has been described in severely infected patients and thus nephrotoxicity cannot be ruled out (1).

In individuals with normal renal function 90% of an administered dose of vancomycin is eliminated through the kidneys (8). In anuric patients adequate serum concentrations can be maintained by about 7% of normal dose (2, 11). The relationship between vancomycin clearance and glomerular filtration rate (GFR) has previously only been investigated in dogs in which the $C_{\text{van}}/C_{\text{cr}}$ ratio was found to be 0.69 ± 0.05 (10).

In anuric patients in intermittent haemodialysis therapeutic concentrations of vancomycin could be maintained with 7% of the normal dose, which is in agreement with previous studies (2, 11).

In patients with varying degrees of renal insufficiency the rate of elimination of vancomycin was dependent on renal function and correlated well with the GFR expressed as $^{250}\text{ml/min}$ creatinine clearance and creatinine clearance. A negative limit correlation was demonstrated between $C_{\text{van}}/C_{\text{cr}}$ ratios and serum albumin concentrations. In a patient with markedly reduced serum albumin concentration the $C_{\text{van}}/C_{\text{cr}}$ ratio was close to 1.0 which means that C_{van} was almost identical with GFR. Since vancomycin can form complexes with peptides in vitro these findings can be explained by partial binding of vancomycin to albumin in the serum and by glomerular filtration of the non-bound vancomycin. Using all data collected C_{van} corresponded to 79%

of the GFR measured by C_{cr} . This may have been due either to binding of vancomycin to serum proteins or reabsorption or backward diffusion of vancomycin in the kidneys. Since studies of binding to serum proteins are not available the present study does not allow any clear-cut conclusion on this question.

It can be concluded that C_{cr} correlates well to the GFR and that the necessary maintenance dose of vancomycin can be calculated on the basis of this value thereby reducing the number of serum vancomycin determinations during treatment. Maintenance dose ($\mu\text{g/day}$) will thus be identical to C_{cr} (ml/min) $\times 1.440$ (min/d) \times therapeutic serum concentration of vancomycin ($\mu\text{g/ml}$) $+ 7\%$ of normal dose ($\mu\text{g/d}$) $- C_{cr} = C_{cr} \times 0.53$ or $C_{cr} \times 0.79$.

In renal insufficient patients vancomycin treatment can be carried out as follows. A normal initial dose is given. Serum vancomycin measurements are necessary during initiation of treatment in order to be sure that a therapeutic level is reached. When the therapeutic level has been obtained the maintenance dose can be calculated on the basis of the above mentioned formula. If a serum concentration of $20 \mu\text{g/ml}$ is desired the maintenance dose will approach $C_{cr} \times 0.53 \times 1.440 \times 20 = (C_{cr} \times 15) \text{ mg/d} + 150 \text{ mg/d}$. In azuric patients 7% of the normal dose will equal maintenance dose per unit time.

REFERENCES

1. Dangerfield H. G. Hewitt, W. L. Monzon, O. T. Kordoff, Z. Blackman B. & Finegold, S. M. Clinical use of vancomycin. In: *Antimicrobial Agents Annual 1960* (ed. P. Gray B. Tabenkin & S. G. Bradley) p. 428. Plenum Press, New York 1961.
2. Etkin, S. Phillips, I. & Evans, J. Vancomycin for staphylococcal shunt site infections in patients on regular haemodialysis. *Brit. med. J.* 3: 80, 1970.
3. Garrod L. P. & O'Grady P. *Antibiotic and chemotherapy*. Churchill & Livingstone London 1972.
4. Geraci, J. E., Heisler F. R., Nichols, H. R. & Wellman W. E. Antibiotic therapy of bacterial endocarditis. VII. Vancomycin for acute micrococc endocarditis. Preliminary report. *Proc. Mayo Clin* 33(7) 172, 1958.
5. Hill R. B. J. Dabring B. E. Stanz T. E. & Rifkind D. Death after transplantation. An analysis of sixty cases. *Amer. J. Med.* 42: 327 1967.
6. Jackson G. G. & Arcieri, G. Ototoxicity of gentamicin in man: a survey and controlled study of clinical experience in the United States. *J. infect. Dis. Suppl.* 124 130, 1971.
7. Jepsen, O. B. Koncentrationssvingninger af vancomycin. *Ugeskr. Læg.* 130: 2174 1968.
8. Kirby W. M. M. & Dineviss, C. L. Vancomycin. Clinical and laboratory studies. In: *Antibiotics Annual 1956-57* (ed. H. Welch & F. Marti-Ibanez) p. 107. Medical Encyclopedia, New York 1957.
9. Kirby W. M. M., Perry D. M. & Bauer A. W. Treatment of staphylococcal septicemia with vancomycin. Report of thirty-three cases. *New Engl. J. Med.* 282: 49 1960.
10. Lee, C.-C., Anderson R. C. & Chen, A. K. Vancomycin, a new antibiotic. V. Distribution, excretion and renal clearance. In: *Antibiotics Annual 1956-57* (ed. H. Welch & F. Marti-Ibanez), p. 11. Medical Encyclopedia, New York 1957.
11. Lindholm D. D. & Murray J. S. Persistence of vancomycin in blood during renal failure and its treatment by hemodialysis. *New Engl. J. Med.* 274 1047 1966.
12. Myerowitz, R. L., Medeiros, A. A. & O'Brien T. F. Bacterial infection in renal homotransplant recipients. A study of fifty-three bacteremic episodes. *Amer. J. Med.* 53 308 1972.
13. Nieto M. & Perkins, H. R. Physicochemical properties of vancomycin and iodovancomycin and their complexes with diacetyl-L-lysyl-D-alanyl-D-alanine. *Biochem. J.* 123 773 1971.
14. - Modifications of the acyl-D-alanyl-D-alanine terminal moiety affecting complex formation with vancomycin. *Biochem. J.* 123 789 1971.
15. Nordström, L., Banck, G., Belfrage S., Juhlin I., Tjernström, Ö. & Torstam, N. H. Prospective study of the ototoxicity of gentamicin. *Acta path. microbiol. scand. Section B Suppl.* 241 38 1973.
16. Riley H. D. Jr. & Ryan H. J. Treatment of severe staphylococcal infections in infancy and childhood with vancomycin. In: *Antibiotics Annual 1959-60* (ed. F. Marti-Ibanez), p. 908. Antibiotics Inc. New York 1960.
17. Skov P. E. Glomerular filtration rate in patients with severe and very severe renal insufficiency. *Acta med. scand.* 187 419 1970.

MONOCLONAL IMMUNOGLOBULINAEMIA ASSOCIATED WITH GLOMERULOPATHY

Herluf Jensen and Allan Wüik

From Medical Department P Division of Nephrology Rigshospitalet the Immunological Laboratory University Clinic for Infectious Disease Blegdamskøpshuset Copenhagen and Medical Department B Section of Nephrology Copenhagen County Hospital Glostrup Denmark

Abstract Four patients with benign monoclonal immunoglobulinaemia and associated glomerulopathy are described. Immunohistochemical investigations of the immunoglobulin-containing cell in the bone marrow revealed an unexpectedly pronounced predominance of monoclonal over polyclonal cells as typically seen in macroglobulinaemia and multiple myeloma, but in contrast to the malignant plasma cell proliferations the percentage of immunoglobulin-containing cells only constituted 6-12% of the nucleated cells. The possible pathogenetic mechanisms relating monoclonal immunoglobulinaemia and glomerulopathy are unknown. The sera did not contain antibodies to glomerular basement membrane, cryoglobulins, antinuclear factors or antiglobulins. The immunohistochemical technique certainly offers clear advantage over conventional bone marrow cytology in the study of patients with monoclonal immunoglobulinaemia.

In 1966 Meltzer et al (10) described the association of essential cryoglobulinaemia and glomerulonephritis in three patients. Since then this combination has been reported by several investigators. In most cases the glomerulopathy has been post-streptococcal glomerulonephritis or rapidly progressive glomerulonephritis of unknown aetiology (1-11).

Monoclonal immunoglobulinaemia mostly without circulating cryoglobulins is found in multiple myeloma, Waldenström's macroglobulinaemia and several other disorders, among which some are termed essential monoclonal immunoglobulinaemia because of lack of knowledge about an underlying cause. The latter disorder has only once been described in combination with glomerulonephritis by Kaplan and Kaplan (7).

In the present paper we report on four patients

with essential monoclonal immunoglobulinaemia and glomerulopathy. Immunohistochemical analysis of the bone marrow was performed in each patient.

MATERIAL AND METHODS

Case history

Patient 1 A 66-year-old male. The family history not contributory. He had passed renal stone 20 years ago. For the last 30 years he had suffered from muscle pain especially in cold weather.

In March 1970 he was admitted to the Medical Department, Rigshospitalet, because of nephrotic syndrome. The proteinuria was 4-6 g/24 h, serum creatinine 1.5 mg/100 ml and endogenous creatinine clearance 79 ml/min. BP was 160/70 mmHg. Serum cholesterol and triglycerides were normal. A kidney biopsy showed membranous glomerulonephritis. Serum contained an IgM- κ M-component in a concentration of 9 g/l determined by paper electrophoresis but no rheumatoid factors, antinuclear factors or cryoglobulins (12). Hb 8 g/l. Bence Jones protein was found in the urine. The bone marrow contained 15% normal plasma cells and 7% lymphoid cells with an irregular nucleus and marrow of strongly basophilic cytoplasm (X-ray) of the skull and pelvis were normal.

Because of the nephrotic syndrome the patient was treated with prednisone 80 mg/day and azathioprine 100 mg/day for 4 weeks without any effect on proteinuria or the concentration of the M-component in serum. The patient was discharged on furosemide therapy. At the last control in March 1974 proteinuria was 3.4 g/24 h, serum creatinine 1.5 mg/100 ml and endogenous creatinine clearance 77 ml/min. BP was 100/100 mmHg on furosemide and methylgluta therapy. Serum concentration of the M-component was 5.3 g/l. The concentration of polyclonal Ig in serum determined by the method of Wacke (14) was normal (Table 1). N hyperviscosity syndrome or other signs of Waldenström macroglobulinaemia were found.

Patient 2 A 53-year-old male. The patient's mother had had diabetes at the age of 60. At the age of 4 a diagnosis of diabetes mellitus was suspected in the patient but no therapy was started. In 1963 proteinuria was found. In 1966 the patient passed 3 renal stones.

In March 1967 he was admitted to the Medical Department Rigshospitalet, because of headache and fatigue. He was obese BP 180/120 mmHg, serum creatinine 2.7 mg/100 ml and endogenous creatinine clearance 49 ml/min. A proteinuria of 5–10 g/24 h was found. Urinary excretion of glucose 0–2 g/24 h. Fasting plasma glucose was normal. A percutaneous kidney biopsy showed lobular glomerulonephritis. Serum contained an M-component of type IgG- κ in a concentration of 13.4 g/l. The same globulin (19% of total urine protein) plus Bence Jones protein was found in the urine. No rheumatoid factors, antinuclear factors or cryoproteins were found in serum. The bone marrow contained only 2% normal plasma cells. X-rays of the skull, pelvis and vertebral spine were normal.

The patient was treated with thiazides and methyldopa and was followed in the Outpatient Department. The kidney function deteriorated and in Nov. 1973 chronic intermittent peritoneal dialysis was started. The concentration of the M-component in serum was at that time unaltered. Serum concentration of polyclonal Ig was normal (Table 1) and there were still no signs of multiple myeloma.

Patient 3 A 46-year-old male. No family history of renal disease, diabetes mellitus or hypertension. At the age of 20 the patient was successfully treated with gold salts for rheumatoid arthritis. At the age of 40 he was treated for a period with glucocorticoids for the same disease. During the last 20 years the patient had consumed large amounts of analgesics because of joint pain. In 1966 proteinuria (2–3 g/24 h) was discovered. Serum creatinine was normal.

In Dec. 1969 the patient was admitted to the Medical Department Rigshospitalet, in terminal anaemia. Serum haemoglobin was 10 mg/100 ml and endogenous creatinine was about 8 mg/min. Proteinuria was 4–5 g/24 h. There were no subjective or objective signs from the joints were found. A kidney biopsy was performed. A histological diagnosis was difficult to establish because of scarring of the kidney tissue, but compatible with chronic proliferative glomerulonephritis. No signs of myelomatous kidney alterations or amyloidosis were found. A biopsy of the rectal mucosa was normal. Serum contained an

M-component of the type IgG- κ in a concentration of 9 g/l. No rheumatoid factors, antinuclear factors or circulating cryoglobulins were found in the serum. The urine contained the same M-component in an amount of 11% of the total protein excretion. No Bence Jones protein was found in the urine. Examination of the bone marrow demonstrated 9% plasma cells, some of which were atypical with vacuolated nuclei and several nuclei per cell. X-rays of the skeleton were without signs of multiple myeloma.

The patient was treated with intermittent dialysis until March 1971 when he received cadaveric kidney. At the time of transplantation the concentration of M-component in serum was 6.2 g/l. Prednisone (initially 150 mg/day) and azathioprine (100–200 mg/day) in combination with extracorporeal irradiation of the blood were given as immunosuppressive treatment.

The course was uneventful and at the last follow-up in April 1974 the patient was doing well with an endogenous creatinine clearance of 75 ml/min. From the time of transplantation until Oct. 1971 (i.e. 7 months) the urine contained no protein, but from this date increased uric acid was seen thus (at the last examination 7.1 g/24 h). The concentration of the M-component in serum was at the time 9.6 g/l. The same M-component was found in urine. The bone marrow contained 8% plasma cells and as in the first bone marrow specimen some of these were atypical. X-rays of the skeleton showed no signs of multiple myeloma. The concentration of polyclonal Ig was normal (Table 1).

Patient 4 A 71-year-old female. Her daughter has chronic kidney disease. For several years the patient had suffered from nocturnal dyspnoea and ECG had shown a WPW block.

In Aug. 1971 the patient was admitted to the Medical Department Copenhagen County Hospital, with a nephrotic syndrome. Proteinuria was 8–12 g/24 h serum creatinine 2.0 mg/100 ml, and endogenous creatinine clearance 38 ml/min. Serum contained an IgM- κ M-component in a concentration of 9 g/l but no rheumatoid factors, antinuclear factors or cryoglobulins. The urine contained no Bence Jones protein. The bone marrow was normal with 5% plasma cells. X-rays of the skull, vertebral spine and extremities were normal. No hyperviscosity syndrome or other signs of Waldenström's macroglobulinaemia were present. A kidney biopsy showed "minimal changes".

The patient was treated with prednisone and had a complete remission of the nephrotic syndrome, and pred-

Table 1 Monoclonal and polyclonal immunoglobulins in serum (g/l) and histological findings in bone marrow

Pat. no.	M-comp	IgG ^a	IgA	IgM	Histological findings in bone marrow
1	2.9	7.6	1.2	0.85	1% normal plasma cell and 5% lymphoid cells
2	13.4	11.2	1.0	0.67	7% normal plasma cells
3	12.0	17.6	0.61	0.88	9% plasma cells, some with vacuolated nuclei
4	10.0	8.6	0.6	1.32	2% normal plasma cells

^aDetermined electrophoretically

^bDetermined immunochemically

plasma was withdrawn in Nov 1971. In May 1973 the patient was admitted in a surgical department and cholecystectomy was performed. During this admission Albusix® was positive but no further examinations were done. The patient was discharged and readmitted in July 1972 with a relapse of the nephrotic syndrome.

Prednisone treatment was resumed this time without success. Proteinuria persisted in an amount of 2-3 g/24 h. Therefore in May 1973 the treatment was supplemented with cyclophosphamide (150 mg a day). In June the same year the urine became free of protein and prednisone was withdrawn in Dec. as was cyclophosphamide in March 1974. At this last follow-up serum creatinine was 1.1 mg/100 ml, endogenous creatinine clearance 41 ml/min, BP was 150/70 mmHg. The urine contained no protein and the urinary sediment was normal. Polyclonal Ig were normal (Table I).

Special investigations

Immunohistochemical study of bone marrow Bone marrow specimens were studied by direct immunofluorescence technique according to the method of Fijnheer et al (13). Unlabelled and fluorescein-isothiocyanate (FITC)-labelled rabbit IgG specific for human α , μ , γ and λ chains were purchased from Dakopatts A/S, Copenhagen. The unlabelled rabbit antibodies were labelled with tetraoxetanylbromamide-isothiocyanate (TMRITC) as described by Cebra and Goldstein (2). Before use all conjugates were tested for specificity by crossed immunoelectrophoresis (8) and by performance tests using monoclonal bone marrow cells from patients with multiple myeloma and macroglobulinaemia (15).

In all patients double staining experiments were done to determine in the same preparation whether the cell containing one particular type of heavy chain also contained only one type of light chain, the essential criterion for calling the plasmacytic proliferation monoclonal.

All cells containing a particular heavy chain type were counted in each cytocentrifuge slide preparation and subsequently the relative percentages were calculated. The κ/λ ratio was always counted in one slide preparation stained with FITC-anti- κ and TMRITC-anti- λ .

Antiglomerular basement membrane antibodies Cryostat sections of normal human kidney tissue (4-6 μ thick) served as substrate for detection of antglomerular basement membrane antibodies by indirect immuno-

fluorescence technique. Sera were screened at dilutions 1:1, 1:4 and 1:16. A 1:50 diluted FITC labelled rabbit antiserum specific for human α , μ , γ and λ chains (Dakopatts A/S, Copenhagen).

RESULTS

In patients 1 and 4 with an IgM κ M-component in serum 83% of the monoclonal bone marrow cells contained μ chains and 96 and 72% respectively contained κ -chains. In patients 2 and 3 with an IgG- κ M-component in serum the corresponding figures were 96, 97 and 98% respectively.

Ig-containing cells as a percentage of nucleated cells were determined in 3 patients and varied from 6.3 to 12.2. In patients 1 and 4 the Ig-containing cells were predominantly lymphoid, and in patients 2 and 3 mainly plasmacytoid (Table II). No antglomerular basement membrane antibodies were found in any of the patients.

DISCUSSION

In addition to patients with multiple myeloma and Waldenström's macroglobulinaemia, monoclonal immunoglobulins are encountered among patients with cancer, amyloidosis and leukaemias. These diseases were excluded on clinical or histological grounds in the present patients.

As monoclonal Ig in plasma can be found in apparently healthy persons, especially in old age (6, 13) there could have been a chance association between benign monoclonal immunoglobulinaemia and kidney disease in our patients. However it is remarkable that all the patients suffered from a glomerulopathy and not from different renal disorders. Furthermore such an association would be rare considering the low incidence of both diseases.

Therefore it is tempting to postulate some kind of

Table II. Immunohistochemically determined relative percentages of bone marrow cells containing γ and μ heavy chains and κ and λ light chains. Morphological feature

Pat no.	Cells containing heavy and light chains (%)					Ig-containing cells 100 nucleated cells	Characteristics of monoclonal cells
	γ	κ	μ	λ			
1	15	2	83	96	4	12.2	Mainly small lymphoid cells with narrow rim of cytoplasm
2	98	1	1	98	2		Mainly plasmacytoid cells
3	97	2	1	98	2	9.0	Mainly plasmacytoid cells
4	12	5	83	72	1	6.3	Both plasmacytoid and lymphoid cells

causal relationship between the monoclonal immunoglobulinaemia and the glomerulopathy. At our present state of knowledge the two pathogenetic mechanisms for the induction of glomerulonephritis should be considered: one involving type II reaction between antiglomerular basement membrane antibodies, glomerular basement membrane and complement leading to damage of the membrane; the other involving type III reaction due to immune complex deposition in the glomeruli. The latter mechanism is thought to be the more common. We cannot say which of the two mechanisms is the most likely in our patients, since no immunohistochemical studies were performed on the renal biopsies. The only earlier report of monoclonal immunoglobulinaemia accompanied by glomerulopathy (7) did not include immunohistochemical studies either. The lack of antiglomerular basement membrane antibody activity in the sera does not exclude type II reactions as such antibodies may be completely trapped in the glomeruli (9).

Serum electrophoresis did not indicate aggregate formation of the M-components, but this does not exclude that the M-component might participate in immune complexes.

Monoclonal immunoglobulinaemia in combination with rheumatoid arthritis has been described by several authors (3-16). The M-component often showed antiglobulin activity. In our rheumatoid arthritis patient (no. 3) such activity of the M-component was not detected. However, the possibility remains that the M-component might have γ activity against circulating autologous IgG.

Finally, the M-component might itself act as foreign antigen, triggering antibody production and immune complex formation. As none of the patients had circulating cryoglobulins the pathogenetic mechanism may be different from that hypothesized by Skrifvars et al. (11).

In the immunohistochemical investigations of the bone marrow the percentage of nucleated cells containing Ig was increased compared to normal persons, but not to an extent found in malignant proliferation of plasma cells or lymphoid cells as seen in multiple myeloma or macroglobulinaemia. However, the monoclonal population constituted a very high percentage of the Ig-containing cells, quite similar to that found as a rule in malignant cell proliferation (4). The last finding compared to the relatively low quantities of

M-component in the corresponding sera suggest that either 1) the monoclonal cells secrete less Ig than polyclonal cells, 2) the M-component is hypercatabolized or excreted very fast, or 3) most of the polyclonal Ig is produced in lymphoid organs outside the bone marrow. At present none of these possibilities can be excluded, although the last one seems the most likely.

The results of conventional histological and immunohistochemical investigation of the bone marrow in this study shows that only the latter investigation gives a true picture of the activity of the bone marrow in producing M-components in monoclonal immunoglobulinaemias.

The experience gained from this study seems to indicate that both the percentage of Ig-containing cells among the nucleated cells and the relative percentage of the Ig-containing cells constituted by M-component-containing cells are relevant in attempting to differentiate between benign and malignant plasma cell proliferations. Obviously longitudinal studies of these parameters are even more important for this distinction.

REFERENCES

1. Barnett E. V. Cryoglobulins and disease. *Ann Intern Med.* 73: 95, 1970.
2. Ceiba J. & Goldstein G. Chromatographic purification of tetramethylrhodamine-immune globulin conjugates and their use in the cellular localization of rabbit γ -globulin polypeptide chains. *J Immunol.* 94: 230, 1965.
3. Dryll A., Ryckewaert A. & Lahn, M. F. Rheumatismes inflammatoires et paraprotéine à propos de neuf observations personnelles. *Rev Rhum.* 34: 155, 1967.
4. Hgman W., Schurt H. R. E. & Helsing-Hersleink, E. An immunofluorescence study on intracellular immunoglobulins in human bone marrow cells. *Ann. N.Y. Acad. Sci.* 177: 290, 1971.
5. Hgman W., Schurt H. R. E. & Klein, F. An immunofluorescence procedure for the detection of intracellular immunoglobulins. *Clin. exp. Immunol.* 4: 457, 1969.
6. Hallén, J. Discrete gammaglobulin (M) components in serum. *Acta med. scand. Suppl.* 462, 1966.
7. Kaplan N. G. & Kaplan, K. C. Monoclonal gammopathy, glomerulonephritis, and the nephrotic syndrome. *Arch. intern. Med.* 125: 696, 1970.
8. Laurell C.-B. Antigen-antibody crossed electrophoresis. *Anal. Biochem.* 10: 358, 1963.
9. McPhaul J. J. & Dixon E. J. The presence of antiglomerular basement membrane antibodies in peripheral blood. *J. Immunol.* 101: 116R, 1969.

- 10 Melzer M, Franklin E. C, Elias H, McCluskey R. T & Cooper N. Cryoglobulinaemia—A clinical and laboratory study. II: Cryoglobulin and rheumatoid factor activity. *Amer J Med* 40: 837 1966.
- 11 Sjöfrans B, Tallqvist G & Torroth T.. Renal involvement in essential cryoglobulinaemia. *Acta med scand* 194: 229 1973.
- 12 Wiger O, Mustakallio K. K. & Räsänen J. A. Mixed IgA-IgG cryoglobulinaemia. Immunological studies and case reports of three patients. *Amer J Med* 44: 179 1968.
- 13 Wälderström J. □ Benign monoclonal gammopathy does. In: *Multiple myeloma and related disorders* (ed H. A. Azar & M. Potter) vol. 1 pp. 347-266. Harper & Row, New York, Evanston, San Francisco and London 1973.
- 14 Weeke B. Quantitative estimation of human immunoglobulins following carbamylation by electrophoresis in antibody-containing agarose gel. *Scand J clin. Lab. Invest.* 22: 107 1968.
- 15 W. K. A. & Maatbe E. Restrictions among heavy and light chain determinants of granulocyte-specific antinuclear factors. *Immunology* 23: 53 1972.
- 16 Zawadzki, Z. A. & Benedek, I. G. Rheumatoid arthritis, dysproteemic arthropathy and para proteinemia. *Arthr and Rheum* L. 445 1969.

Medical Journals

Printed and distributed by Almqvist & Wiksell
publishers to the Universities of Uppsala, Stockholm and G
and to the Royal Swedish Academy of Science, etc

Acta Chirurgica Scandinavica

Editor: L. Thoren

8 issues per volume. Free supplements. Including free subscriptions to the *Scandinavian Journal of Plastic and Reconstructive Surgery* (without suppl.), the *Scandinavian Journal of Thoracic and Cardiovascular Surgery* (without suppl.) and the *Scandinavian Journal of Urology and Nephrology* (without suppl.)

Current volume 141/1975

Sw kr 50 per volume, incl. postage

Acta Dermato-Venereologica

Editor: Nils Thyreman

6 issues per volume. Free supplements.

Current volume 55/1975.

Sw kr 130 per volume, incl. postage

Acta Medica Scandinavica

Editor: J. Waldenström

6 issues per volume. Free supplements.

Current volumes 197-198/1975

Sw kr 225 per annum (two volumes) incl. postage

Acta Obstetrica et Gynecologica Scandinavica

Editor: Axel Fogelmark-Sundberg

5 issues per volume. Free supplements.

Current volume 54/1975

Sw kr 150 per volume, incl. postage

Acta Oto Laryngologica

Editor: C.-A. Hamberger

6 issues per volume. Free supplements

Current volumes 79-80/1975

Sw kr 100 per volume. Two volumes per annum

Sw kr 200, incl. postage

Pædiatriska Scandinavica

R. Zetterstrom

per volume. Free supplements

volume 64/1975

Sw kr 175 per volume, incl. postage

International Journal of Fertility

Editor: S. J. Behrman

4 issues per volume

Current volume 20/1975

Sw kr 120 per volume, incl. postage

International Journal of Gynaecology and Obstetrics

Editor: Harold A. Kamnitsky

6 issues per volume.

Current volume 13/1975

Sw kr 110 per volume, incl. postage

Scandinavian Audiology

Editor: Björn Blegvad

4 issues per volume. Free supplements.

Current volume 4/1975

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Infectious Diseases

Editors: Justus Ström and Sten Wihlbjörk

4 issues per volume. Free supplements.

Current volume 7/1975

Sw kr 110 per volume, incl. postage

Scandinavian Journal of Plastic and Reconstructive Surgery

Editor: Bengt Johansson

3 issues per volume. Free supplements.

Current volume 9/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Psychology

Editor: Lars Leeborn

4 issues per volume

Current volume 16/1975

Sw kr 98 per volume, incl. postage

Scandinavian Journal of Rehabilitation Medicine

Editor: Olle Hock

4 issues per volume. Free supplements.

Current volume 7/1975

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Rheumatology

Editor: Velkko Laine

4 issues per volume. Free supplements.

Current volume 4/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Social Medicine

Editor: Gunnar I. gbe

3 issues per volume. Free supplements.

Current volume 3/1975.

Sw kr 100 per volume incl. postage

Scandinavian Journal of Thoracic and Cardiovascular Surgery

Editor: Viking Olav Björk

3 issues per volume. Free supplements

Current volume 9/1975

Sw kr 100 per volume incl. postage

Scandinavian Journal of Urology and Nephrology

Editor: Åke Frykholm

3 issues per volume. Free supplements.

Current volume 9/1975

Sw kr 100 per volume, incl. postage

Uppsala Journal of Medical Sciences

Editor: Gunna Ågren

3 issues per volume. Current volume 80/1975

Sw kr 70 per volume, incl. postage

Free inspection copies on request—write to

The Almqvist & Wiksell Periodical Company
Box 62, S 101 20 Stockholm Sweden

THE EFFECT OF NOREPINEPHRINE AND THEOPHYLLINE ON BLOOD GLUCOSE PLASMA FFA PLASMA GLYCEROL AND PLASMA INSULIN IN NORMAL SUBJECTS

Krister Arman, Sven Carlström and Jan I. Thorell

From the Department of Internal Medicine A, University Hospital, Lund, and the Isotope Laboratory, Malmö General Hospital, Malmö, Sweden

Abstract The plasma concentrations of free fatty acids (FFA), glycerol and insulin as well as the blood glucose concentrations have been followed in two groups of subjects after infusion of theophylline. Each individual was examined twice. The 5 subjects in group I were given an infusion of norepinephrine before the theophylline at one of the examinations and saline at the other. The 6 subjects in group II were given an infusion of norepinephrine at both examinations, followed by theophylline on one occasion and by saline on the other. Thus, the subjects in both groups served as their own controls. It was found that theophylline caused lipid mobilization, as measured by the plasma FFA and plasma glycerol concentrations, both when given as the only active drug and when given after norepinephrine. The blood glucose concentration rose slightly after norepinephrine and the plasma insulin level increased concomitantly. When theophylline was given as the only active drug, there was no increase in the blood glucose but the plasma insulin concentration rose slightly.

Theophylline has been shown to exert a stimulating effect on the lipolytic process in adipose tissue in animals (2) and on human adipose tissue in vitro (3).

According to the current theory the lipolytic effect is mediated through inhibition of the phosphodiesterase activity which enzyme converts cyclic AMP (cAMP) to inactive AMP. Theophylline, thus, increases the cAMP concentration by diminishing its breakdown and as the cAMP concentration controls the rate of lipolysis consequently stimulates lipolysis.

The catecholamines, on the other hand, exert an effect on lipolysis by stimulation of the adrenergic receptors in adipose tissue causing an activation of the enzyme adenylcyclase, which converts ATP to cAMP. As a result, the concentration of cAMP is

increased and lipolysis stimulated. Thus theophylline and catecholamines both increase the cAMP concentration, but their effects are mediated via separate enzymatic mechanisms and it is theoretically possible to expect an additional effect when both drugs are given.

The present study was started as a trial to elucidate 1) if theophylline in vivo has an effect on the lipolysis and the lipid mobilization, as measured by the plasma free fatty acid (FFA) and the plasma glycerol concentrations and 2) if theophylline in vivo further increases the lipid mobilization induced by norepinephrine. Preliminary results of this study have been published earlier (5).

MATERIAL AND METHODS

Eleven apparently healthy male volunteers, aged 23-44 years, were studied. The subjects were divided into group I ($n=5$ mean age 27 years) and group II ($n=6$ mean age 30 years) and each individual was examined on two occasions on different days.

The examination started in the morning with the subjects in the fasting state. One catheter was inserted into the brachial artery and one into one of the cubital veins. After about one hour' rest infusions of the drugs were given via the venous catheter and blood samples were drawn through the arterial catheter. The drugs were infused during two consecutive periods of 10 min each; hereafter the subjects rested for 60 min.

The subjects in group I were on one occasion given 75 µg norepinephrine during the first 10 min and 10 ml theophylline (ACO (theophylline 230 mg/ml) during the following 10 min. On the other occasion the same individuals were given physiological saline during the first period and the theophylline during the second period. Thus, the individuals served as their own controls.

In group II the subjects were given 75 µg norepinephrine

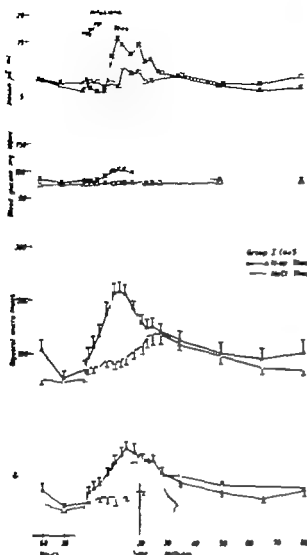


Fig. 1 Plasma FFA, plasma glycerol, blood glucose and plasma insulin concentrations during the experiment in group I. Mean \pm S.E.M.

during the first infusion period on both occasions. It was followed by 10 ml theophyllamine ACO, the second infusion on one occasion and by physiological saline on the other. In this group too, the subjects served as their own control.

During the whole experimental period ECG was recorded and direct measurements of the intrarterial BP were made at intervals. Blood samples were drawn at 6 and 30 min before, at the start of and at 1, 3, 5, 8, 11, 13, 15 and 18 min during the infusions. After the infusions blood samples were drawn at 1, 23, 25, 28, 35, 40, 65 and 80 min. The blood was collected in test tubes containing heparin as anticoagulant.

The blood samples were kept on ice water and plasma was separated within 90 min. The whole experiment was performed in the Department of Clinical Physiology, University Hospital of Lund, Sweden.

Plasma FFA was titrated according to Trout et al. (13). Plasma glycerol was determined according to Laurell and Tibbling (9) and blood glucose according to Mark (11). Plasma insulin was estimated with the method of Hedin (7).

RESULTS

The plasma FFA, plasma glycerol, blood glucose and plasma insulin concentrations in group I during the experiment are illustrated in Fig. 1. It is apparent that the norepinephrine and theophylline infusions increased the concentrations of plasma FFA, plasma glycerol and blood glucose. The plasma insulin level rose slightly, parallel to the increase in blood glucose.

When the individuals in group I were given saline as the first infusion, the following theophylline infusion caused a rise in the plasma FFA and the plasma glycerol, blood glucose and plasma insulin concentrations in group II. When norepinephrine was given, it showed no variations. The plasma insulin level rose slightly but significantly.

Fig. 2 shows the mean plasma FFA, plasma

Table 1 Hemodynamic data

Combinations of infusions	Before infusion				During infusion I				During infusion II				After infusion			
	BP				BP				BP				BP			
	S	D	M	HR	S	D	M	HR	S	D	M	HR	S	D	M	HR
Group I (n=5)																
N-ep+Theo	116	71	91	60	143	83	108	48	123	68	90	64	122	73	83	61
NaCl+Theo	114	67	85	52	116	66	85	50	122	71	90	56	116	73	96	61
Group II (n=6)																
N-ep+Theo	109	68	82	56	135	69	103	49	114	66	84	60	115	64	87	58
N-ep+NaCl	111	65	83	53	131	78	100	46	115	62	83	55	114	67	83	56

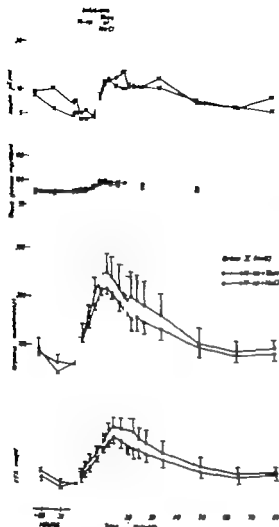


Fig. 2 Plasma FFA, plasma glycerol, blood glucose and plasma insulin concentration during the experiment in group II. Mean \pm S.E.M.

glycerol, blood glucose and plasma insulin concentrations in group II. When norepinephrine was given as the only active drug, followed by saline, there were rises in plasma FFA, plasma glycerol and blood glucose of the same magnitude as in group I with the same infusions. When however the norepinephrine infusion was followed by theophylline, the rises in plasma FFA and plasma glycerol were somewhat more pronounced, lasted longer and showed tendency towards two maxima.

The blood glucose curve was the same on both occasions in group II. Both curves showed a slight rise and a concomitant slight decrease in the plasma insulin level.

The hemodynamic data are summarized in Table I. In group I the mean intraarterial BP rose and the heart rate decreased when norepinephrine was given as the first infusion. When saline was given, no variations were noticed. The subjects in group II showed on both occasions a rise in the mean arterial BP and a bradycardia following the norepinephrine infusion.

The theophylline infusion caused no variations in the hemodynamic parameters in either group.

DISCUSSION

These studies indicate that theophyllamine is an active stimulator of lipolysis and lipid mobilization *in vivo* when measured as the plasma concentrations of FFA and glycerol. The response to theophyllamine was delayed as compared to the norepinephrine response. This supports the view of dissimilar mechanisms for the effect of norepinephrine and theophyllamine on the lipolysis. If one accepts the theory that their effects are related to an increased production rate of adenylylase by the former and a decreased degradation rate by the latter, one would expect the combined use of both agents to result in exaggerated responses regarding FFA and glycerol. This was the case and furthermore, these combined responses had a 2-phase character as if the two stimulants worked in an unrelated manner.

Theophyllamine given as the only drug induced a small rise in insulin in spite of the absence of changes in blood glucose. It is known that agents which raise the concentration of cAMP in the B cell enhance the glucose-stimulated release of insulin (10), though this effect is not seen in the absence of glucose. But the marked increase in FFA may have exerted an influence as well. It has been reported that experimental elevation of FFA in dogs stimulates the release of insulin (6). In man FFA alone does not stimulate to increased insulin secretion (12) but may enhance the response to other stimuli (1). Combined enhancing effects of cAMP and FFA may therefore cause a sensitization of the B cells for glucose to the extent that the basal concentration of glucose is high enough to induce an increased release of insulin. The even higher insulin response noted in the norepinephrine experiments when the glucose level rose slightly is then probably just an exaggeration of the same effect.

The lipolytic response as well as the dynamic data when norepinephrine

as the only active drug, were in agreement with earlier findings (4). Theophylline showed no hemodynamic effects.

Theophylline is commonly used in the treatment of asthma bronchiale and cardiac insufficiency. Judging from the present results it is able to cause an elevation of the plasma FFA concentration. Kamen et al. (8) have proposed that raised plasma FFA levels may induce arrhythmias in patients with myocardial infarction. If their theory is correct, theophylline should perhaps be avoided in the treatment of cardiac insufficiency in patients with myocardial injury.

ACKNOWLEDGEMENTS

Financial support was obtained from the Swedish Medical Research Council (grants no. K73-19X 3784 and 13X 3506), the Medical Faculty University of Lund and the National Swedish Association against Heart and Chest Diseases.

REFERENCES

1. Balise E. G. & Ooms, H. A.: Role of plasma free fatty acids in the control of insulin secretion in man. *Diabetologia* 9: 145 1973.
2. Brodie, B. B., Davies, J. I., Hyne, S., Krishna, G. & Wiers, B.: Interrelationships of catecholamines with other endocrine systems. *Pharm. Rev.* 18: 773 1966.
3. Carlsson, L. A., Hallberg, D. & Micéll, H.: Quantitative studies on the lipolysis response of human adipose tissue to noradrenaline and theophylline. *Acta med. scand.* 185: 465 1969.
4. Carlström, S.: Studies on fatty acid metabolism in diabetics during exercise. VI. Infusions of norepinephrine to male non-insulin treated juvenile diabetics. *Acta med. scand.* 182: 513 1967.
5. Carlström, S. & Thorell, J.: The effect of norepinephrine and theophylline on blood glucose, plasma FFA, plasma glycerol and plasma insulin in normal subjects. *Excerpta med. International Congress Seriet No. 280-B* 1973.
6. Crespen S. R., Greenough III W. B. & Steinberg, H.: Stimulation of insulin secretion by infusion of free fatty acids. *J. clin. Invest.* 48: 1934 1969.
7. Heding, L.: A simplified insulin radioimmunoassay method. In: *Labeled protein in tracer studies* (ed. L. Danato, G. M. Heud & F. Sacchi) pp. 345-351. *Euratom* 1966.
8. Kurica, V. A., Yates, P. A. & Oliver, M. F.: Free fatty acids, heparin and arrhythmias during experimental myocardial infarction. *Lancet* 185 1969.
9. Laurell, S. & Tibblin, G.: An enzymatic fluorometric micromethod for the determination of glycerol. *Chim. chim. Acta* 13: 317 1966.
10. Malaisse, W. J., Malaisse-Lagae, F. & Mayhew, D.: A possible role for the adenylylase system in the insulin secretion. *J. clin. Invest.* 46: 1774 1967.
11. Marks, V.: An improved glucose-oxidase method for determining blood, CSF and urine glucose levels. *Chim. chim. Acta* 4: 395 1959.
12. Schalch, D. S. & Kipolis II M.: Abnormalities in carbohydrate tolerance associated with elevated plasma nonesterified fatty acids. *J. clin. Invest.* 44: 2010, 1965.
13. Trout II L., Estes, E. H. Jr & Friedberg, S. J.: Titration of free fatty acids of plasma: A study of current method and a new modification. *J. Lipid Res.* 1: 199 1960.

PORTAL AND CUBITAL SERUM INSULIN DURING ORAL, PORTAL AND CUBITAL GLUCOSE TOLERANCE TESTS

B Lund, A Schmidt and T Deckert

*From Medical Departments of Steno Memorial Hospital Gentofte
and Copenhagen City Hospital, Copenhagen, Denmark*

Abstract Oral intracubital and intraportal glucose tolerance tests have been performed on 7 non-obese non-diabetics, and glucose and insulin concentrations have been followed in the peripheral and portal blood. A significant rise in portal glucose and insulin was found 1-2 min after oral glucose intake. There was no lag between the rise in insulin and glucose concentrations. The portal glucose concentration after oral glucose intake was significantly higher than after cubital glucose infusion for 45 min, although the peripheral glucose concentrations were identical. In the cubital vein the insulin concentration after oral glucose intake was significantly higher than after intracubital glucose infusion, but in the portal blood there was no difference. After portal glucose infusion the cubital insulin concentration did not differ significantly from the concentration after intracubital glucose infusion. Thus, it seems likely that a high portal glucose concentration is responsible for the higher peripheral insulin concentration after oral glucose intake. A high portal glucose concentration does not seem to influence the hepatic uptake or release of glucose.

Oral intake of α -glucose is known to be followed by a stronger insulin response than a corresponding i.v. glucose infusion (13) but it is not clear which factors are responsible for the difference. Various intestinal hormones—gastric inhibitory peptide (5), secretin (4), gastrin (18), enteroglucagon (15, 22) and insulin-releasing polypeptide (21)—have been suggested, but nervous stimuli (8, 16, 20) and hepatogenic factors (7) have been mentioned as well.

The present study was designed to investigate whether hepatogenic factors play a role in the potentiated insulin response in man following oral intake of glucose. In other words whether high portal glucose concentration is able to increase the secretion or decrease the elimination of insulin.

It has been demonstrated by Goetz et al. (7) that

intraportal infusion of glucose to the dog increases the insulin secretion without any change in the glucose concentration in the systemic arterial blood indicating an extrapancreatic glucose-sensitive regulatory mechanism. Kaplan and Madison (10) found hepatic extraction of insulin ¹²⁵I to fall with its ng glucose concentration in the liver indicating a reduced hepatic elimination of insulin at high portal glucose concentrations.

We therefore studied glucose and insulin concentrations in the portal and cubital vein of non-diabetics during oral intracubital and intraportal glucose tolerance tests (GTT).

MATERIAL AND METHODS

The material comprises nine patients, tested on 3 different days, 5-10 days after laparotomy involving cholecystectomy or seven and exploratory operations because of pain in two. Seven of the patients (6 females and 1 male) were non-diabetics and non-obese and 31-66 years (mean 53). Two patients were immediately scheduled as their blood sugar levels exceeded 150 mg/100 ml 120 min after oral administration of glucose. During the operation a baby feeding tube was inserted into the portal vein through the obliterated umbilical vein. The catheter was kept open by constant infusion of heparinized saline. At the time of the investigations all the patients were fully mobilized and had normal intestinal, hepatic and renal function.

On the first test day the patients received 50 g α -glucose by mouth after 12 hours fasting. Blood samples were drawn simultaneously from the cubital vein, portal vein, and ear capillaries. Capillary plasma glucose, venous serum glucose from the cubital and portal veins as well as endogenous serum insulin from the cubital and portal veins were determined at the times shown in Figs 1-4.

On the second test day glucose infusion was given through cubital vein, and the same parameters were investigated.

On the third test day glucose infusion was given

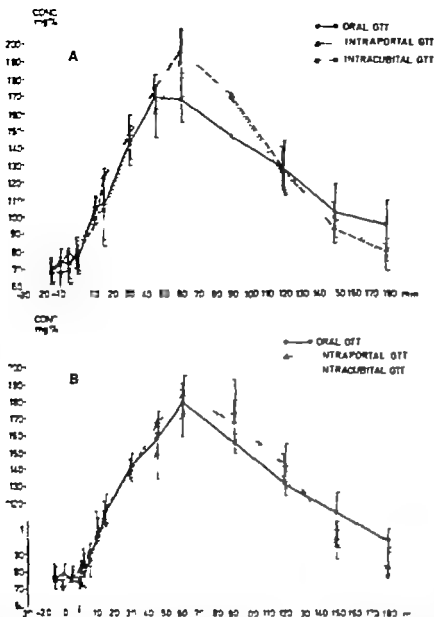


Fig. 1 Plasma glucose in capillary (A) and cubital blood (B) (mean \pm S.E.M.)

through the portal venous catheter. Simultaneous blood sampling for determining portal glucose and portal insulin was not possible, but otherwise glucose and insulin were determined as on the first test day. The glucose infusion rate was adjusted so that the glucose concentrations in the peripheral blood were comparable to those obtained with the oral glucose load.

On all blood samples from the portal vein, haematocrit was determined in order to dilution from the saline infusion. Oxygen saturation in the portal blood was determined periodically as a relative measure of the flow variations of the portal blood. Determination of glucose was performed by the glucose oxidase method on Beckman's auto-analyser. Immunoreactive insulin was determined by the solid phase immunoassay technique of Wade (24). The intra-assay coefficient of variation was 8.5%; inter-assay coefficient of variation 12.4%. The dif-

ference between insulin and glucose concentration during the various tolerance tests was assessed by Student's *t*-test for paired data.

RESULTS

Fig. 1 gives the plasma glucose concentrations in ear capillary blood and in cubital blood during the oral, intracubital and intraportal GTT. It is evident that during the *iv* loads it was possible to attain glucose concentrations of the same course as those during the oral glucose loads.

From Fig. 1 it is apparent that although the glucose concentration peripherally (in ear blood and in the cubital vein) did not differ significantly during

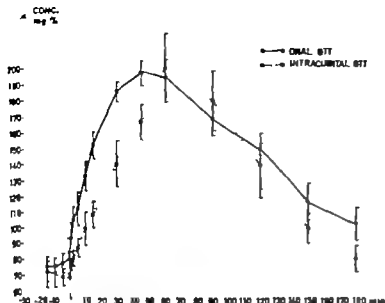


Fig. 2 Plasma glucose in portal blood following oral and intracubital glucose load (mean \pm S.E.M.).

the oral and the i.v. glucose loads (Fig. 1) there was a considerable difference from the glucose concentration in the portal vein. In the portal blood the glucose concentration rose abruptly immediately after the oral intake of glucose, and as early as 3 min after the intake it had increased by 34 mg/100 ml. The portal glucose concentration during this test proved to be significantly higher for up to 45 min than during the cubital test (Fig. 2).

The mean insulin concentration in serum from

the cubital and portal veins during the tests is shown in Figs. 3 and 4. From Fig. 3 it is apparent that the insulin concentration in the cubital vein was significantly higher after oral than after intracubital administration of glucose.

Cubital vein insulin concentration during intraportal glucose infusion showed marked variations. In patients showing almost complete agreement between the glucose concentration peripherally during the course of all three tolerance tests

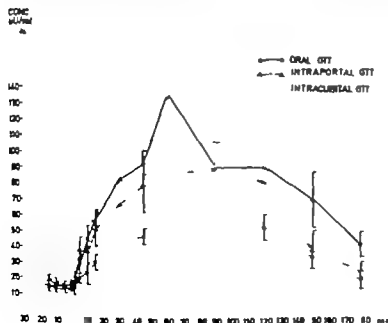


Fig. 3 Plasma insulin in cubital blood following oral, intraportal, and intracubital glucose loads (mean \pm S.E.M.). *—significantly different values following oral and intracubital administration of glucose.

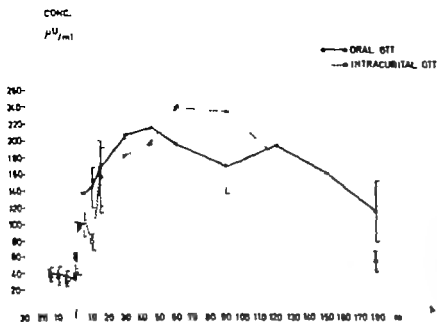


Fig. 4 Plasma insulin in portal blood following oral and intracubital glucose load (mean \pm S.E.M.).

the cubital vein insulin concentration in the portal tolerance test was identical with that during the cubital tolerance test and lower than that during the oral test (Table 1). In others the cubital vein insulin concentration was on a few occasions higher during the portal than during the oral load. These high values were invariably preceded by higher peripheral glucose concentrations. On an average the insulin concentration in the cubital vein was higher following portal than following cubital infusion of glucose. However the difference was not significant. The insulin concentration in the cubital vein following portal infusion of glucose was also not significantly lower than that following oral administration of glucose.

Fig. 4 sets out the insulin concentrations in the portal blood during i.v. and oral glucose loads. Unlike the findings in the cubital vein there was no difference in the insulin concentration in portal blood apart from the 10 min after the commencement of the infusion which marks the well known biphasic insulin response during infusion of glucose.

DISCUSSION

The object of the procedure was to attain a uniform glucose concentration around the β -cells after the different routes of administration in order thereby to secure a uniform glucose-induced insulin response. From Fig. 1 (A) it may be seen that the

Table 1 Plasma glucose and insulin in two patients with nearly identical glucose concentrations during the glucose infusions

Time (min)	Glucose concentration in capillary blood (mg/100 ml)						Insulin in cubital plasma (μ U/ml)					
	0	15	30	45	60	120	0	15	30	45	60	120
Patient 3												
Oral glucose	79	114	146	186	178	110	10	43	39	133	90	60
Intravenous glucose	72	96	135	185	10	148	79	16	3	29	31	40
Intraportal glucose	82	112	158	199	215	147	10	13	28	33	34	4
Patient 5												
Oral glucose	79	115	139	175	198	136	16	63	99	103	75	94
Intravenous glucose	85	123	152	164	226	150	16	41	48	57	70	79
Intraportal glucose	87	122	160	190	230	178	16	30	41	47	58	105

mean plasma glucose concentration in the capillaries of the earlobe did not differ after the different forms of administration. Provided the blood flow in the capillaries of the inlet tissue does not alter its direction with the different modes of administration the capillary plasma glucose concentration around the β -cells must also be considered to have been comparable after different routes of administration. Accordingly a difference in the cubital serum insulin concentration must be due to non-glucose-induced changes in the secretion or/and elimination of insulin. Fig. 4 shows that the insulin concentration in the portal vein during oral and cubital GTT did not differ although the cubital insulin concentration following oral administration was significantly higher than after cubital glucose infusion. This might indicate the same insulin secretion during both tolerance tests but a reduced elimination of insulin in the liver during the oral glucose load.

It is possible, however, that the blood flow in the portal vein during oral glucose load is greater than during an i.v. glucose load. At least it has been demonstrated by Arpoult et al. (1) that the splanchnic flow in dogs increases during oral glucose load. Kaden et al. (9) however found an unchanged portal blood flow in dogs following intraduodenal administration of glucose. During an i.v. glucose infusion the blood flow in the pancreas does not alter (12). We were unable to measure the blood flow in the portal vein in our experimental design but measurement of the oxygen saturation of portal blood showed no systematic variations (85-91% after all routes of administration). Accordingly we are unable to answer the question whether insulin secretion during oral glucose load is increased or unchanged as compared with insulin secretion during the cubital glucose load but we considered it most likely that the portal blood flow is increased during oral glucose intake. However an unchanged portal insulin concentration at an increased flow in the portal vein during oral glucose load must signify an increased secretion of insulin.

Whether elimination of insulin is reduced during oral glucose intake is a question for future studies. Genuth (6) was unable to demonstrate changes in insulin elimination during i.v. glucose tolerance tests and Kaden et al. (9) demonstrated an unchanged or increased insulin elimination in dogs during duodenal GTT. There are no accessible reports elucidating the elimination of insulin in man

during oral glucose loads. Kaplan and Madison's studies (10) have been criticized (9).

Thus, while it is likely that insulin secretion during oral glucose loads is greater than during cubital glucose loads it has not been elucidated whether high portal glucose concentration plays any role in this connection.

The present study has shown that the portal glucose concentration is significantly higher during oral than during cubital administration of glucose. Therefore it cannot be rejected that a high portal glucose concentration is responsible for the difference in cubital insulin concentration observed after oral and i.v. administrations respectively. To prove or disprove this the above mentioned portal glucose infusions were performed, in which the amount of glucose and rate of infusion were identical with those of the cubital glucose infusion. It was not technically possible to measure the portal glucose concentration during the portal infusion of glucose but it must be assumed that this was considerably higher than during the cubital infusion. However it cannot be decided whether it was also higher than during the oral glucose load but this must be considered likely.

A high portal glucose concentration such as that obtained during portal infusion of glucose did not, however, result in a significantly higher cubital insulin concentration (Fig. 3). This agrees with studies in dogs by McIntyre et al. (14) who found identical insulin concentrations peripherally after portal and after peripheral glucose infusion. White and Dupré (13) too have demonstrated with a few case reports that portal glucose infusion does not lead to a higher cubital insulin concentration than does cubital infusion of glucose.

The cubital insulin concentration during portal infusion of glucose was not, however, significantly lower than during oral glucose load in our studies. True, the individual results varied widely as it was not possible to attain identical capillary plasma glucose concentrations in all the subjects during the different routes of administration. But if the successful tests are considered separately (Table I) it is striking that the cubital insulin concentration during portal infusion coincided with this concentration during cubital infusion but not with the concentration during oral administration.

It is unlikely therefore that the potentiated insulin response following oral administration is caused due to mechanisms elicited by

glucose concentration. Gastrointestinal hormones or neurogenic mechanisms elicited from the gastrointestinal tract must still be considered the most important factors in potentiating insulin secretion following oral administration of glucose. This appears even more likely as potentiation of insulin secretion follows not only upon oral administration of *glucose* but also upon oral administration of amino acid (17) and fat (2). According to the findings made so far the gastrointestinal hormone which seems the most likely candidate for potentiating insulin secretion during oral GTT is the gastric inhibitory peptide (GIP) as injection of GIP causes an increase in insulin concentration and as the serum GIP concentration rises during oral but not during i.v. GTT (5). However it cannot be ruled out that other gastrointestinal hormones are operative too.

Neurogenic mechanisms elicited from the intestinal canal are not likely to play a major role in man considering that Lund et al. (11) found the same potentiating effect of orally administered glucose upon the insulin response in patients before and after total vagotomy.

Lastly an unintended result of the present study must be pointed out because it concerns a problem which has been widely discussed. This is the question whether the portal glucose concentration alone is able to regulate the glucose uptake and/or glucose release in and from the liver independently of variations in the insulin and glucagon concentrations. The finding that the capillary plasma glucose concentration is the same after portal and after cubital infusion of glucose indicates that the greater amount of glucose presented to the liver during the portal infusion of glucose does not alter the regulatory mechanisms of the liver in man. Similar findings have been made by McIntyre et al. (14) in the dog. Thus whereas the liver does not seem to exhibit any autoregulation in the case of major acute changes in plasma glucose, it cannot be ruled out that the liver exerts an autoregulatory function on minor variations in blood glucose (3, 19).

REFERENCES

1. Aronold Y., Beßens, R., Conrad, V., Franckson J. R. M. & Mauguier, P. Influence of the intestinal absorption of glucose on the major components of the glycoregulation in anesthetized dogs. *Metabolism* 11: 166 1965.
2. Carroll A. F. & Nestel, P. J. Effect of long-chain triglyceride on human insulin secretion. *Diabetes* 21: 923 1972.
3. Deckert, T. & Egg, P.. Variation in plasma glucose in normal subjects and diabetics in the fasting state. *Acta med. scand* 181: 331 1970.
4. Dupré J. J., Cortis D. & Beck, J. C. Effects of digestive secretagogues on the endocrine pancreas in man. *Diabetes* 1st: Proceedings of the sixth Congress of the International Diabetes Federation in Stockholm 1967 (ed J. Östman) pp. 447-445. Excerpta Medica Foundation, Amsterdam 1969.
5. Dupré J. J., Ross S. A., Watson, D. & Brown, J. C. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J. clin. Endocr.* 37: 826 1973.
6. Genuth S. M. Metabolic clearance of insulin in man. *Diabetes* 21: 1003 1972.
7. Goetz, F. S., Maney J. W. & Greenberg, B. Z. The regulation of insulin secretion. Effects of the infusion of glucose, ribose and other sugars into the portal vein of dogs. *J. Lab. clin. Med.* 69: 537 1967.
8. Immen, J. Effect of acetylcholine on the secretion of glucagon and insulin from the isolated perfused canine pancreas. *Diabetes* 11: 381 1973.
9. Kaden M., Harding, Ph. & Field J. B. Effect of intraduodenal glucose administration on hepatic extraction of insulin in anesthetized dog. *J. clin. Invest.* 52: 7016 1973.
10. Kaplan M. & Madson L. L. Effects of endogenous insulin secretion on the magnitude of hepatic binding of labelled insulin during a single transhepatic circulation in human subjects. *Clin. Res.* 7: 48 1959.
11. Lund B., Aagaard P. & Deckert T. Effect of vagotomy on insulin release after oral and intraduodenal glucose administration. To be published.
12. Martins E. B., Gander L., Seydoux J., Wolfheim, C. B., Kanaza A., Y. Orci, L., Renold A. & Porte D. Glucagon release induced by pancreatic nerve stimulation in the dog. *J. clin. Invest.* 52: 1: 46 1973.
13. McIntyre N., Holdsworth C. D. & Turner D. S. New interpretation of oral glucose tolerance. *Lancet* 2: 20 1964.
14. McIntyre N., Turner D. S. & Holdsworth C. D. The role of the portal circulation in glucose and fructose tolerance. *Diabetologia* 6: 593 1970.
15. Moody A. J., Marcussen, J., Schach Fries, A., Steenstrup C., Sundby F., Malmø W. & Malmøe Lague F. The insulin releasing activities of extracts of pork intestine. *Diabetologia* 6: 135 1970.
16. Port D., Gander L., Seydoux J., Kanaza A. & Posternak, J. Neural regulation of insulin secretion in the dog. *J. clin. Invest.* 52: 10 1973.
17. Rapt, S., Dollinger H. C., Schröder A. E., Schleyer M., Rothenbuchner G. & Pfeiffer F. F. Differences in insulin growth hormone and pancreatic enzyme secretion after intravenous and intraduodenal administration of mixed amino acid in man. *New Engl. J. Med.* 288: 1199 1973.
18. Rehfeld J. F. & Stadil F. The effect of gain on basal- and glucose-stimulated inulin secretion in man. *J. clin. Invest.* 51: 1415 1973.
19. Rodeman, N. B. & Herrera, M. G. Glucose regula-

- tion of hepatic glycoseogenesis. *Amer J Physiol* 214 1346 1968
- 20 Sharp R., Culbert, S. Cook, J. Jennings A. & Burr I. M. Cholergeric modification of glucose-induced biphasic insulin release in vitro. *J clin Invest* 53 710 1973
- 21 Trier D. & Marks S. V. Enhancement of glucose-stimulated insulin release by an intestinal polypeptide in rats. *Lancet* i 1095 1972.
- 22 Unger R. H. Obiedo, A. Valverde I. Eisenman A. M. & Exton, J. Characterization of the responses of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. *J clin Invest* 47 48, 1968
- 23 White, J. J. & Dupré J. Regulation of insulin secretion by the intestinal hormone secretin. Studies in man via transumbilical portal vein catheterisation. *Surgery* 64 204 1968
- 24 Wide C. Radioimmunoassays employing antisera to bombesin. *Acta endocr (Kbh.), Suppl.* 142 207 1969

glucose concentration. Gastrointestinal hormones or neurogenic mechanisms elicited from the gastrointestinal tract must still be considered the most important factors in potentiating insulin secretion following oral administration of glucose. This appears even more likely as potentiation of insulin secretion follows not only upon oral administration of glucose but also upon oral administration of amino acid (17) and fat (2). According to the findings made so far the gastrointestinal hormone which seems the most likely candidate for potentiation of insulin secretion during oral GTT is the gastric inhibitory peptide (GIP) as injection of GIP causes an increase in insulin concentration and as the serum GIP concentration rises during oral but not during i.v. GTT (5). However it cannot be ruled out that other gastrointestinal hormones are operative too.

Neurogenic mechanisms elicited from the intestinal canal are not likely to play a major role in man considering that Lund et al. (11) found the same potentiating effect of orally administered glucose upon the insulin response in patients before and after total vagotomy.

Lastly an unintended result of the present study must be pointed out because it concerns a problem which has been widely discussed. This is the question whether the portal glucose concentration alone is able to regulate the glucose uptake and/or glucose release in and from the liver independently of variations in the insulin and glucagon concentrations.

The finding that the capillary plasma glucose on is the same after portal and after cubital infusion of glucose indicates that the greater amount of glucose presented to the liver during the portal infusion of glucose does not alter the regulatory mechanisms of the liver in man. Similar findings have been made by McIntyre et al. (14) in the dog. Thus, whereas the liver does not seem to exhibit any autoregulation in the case of major acute changes in plasma glucose it cannot be ruled out that the liver exerts an autoregulatory function on minor variations in blood glucose (3, 19).

REFERENCES

1. Arnould Y, BaBens R, Comard V, Franchison J R. M. & Malgouet P. Influence of the intestinal absorption of glucose on the major components of the glycoregulation in anesthetized dogs. *Metabolism* 14: 166, 1965.
2. Carroll K. F. & Nestel P. J. Effect of long-chain triglyceride on human insulin secretion. *Diabetes* 21: 923, 1972.
3. Deckert T & Egg P. Variation in plasma glucose in normal subjects and diabetics in the fasting state. *Acta med. scand.* 181: 331, 1970.
4. Dupré J J, Curtis D & Beck J C. Effects of digestive secretagogues on the endocrine pancreas in man. *Diabetes*. In: Proceedings of the sixth Congress of the International Diabetes Federation in Stockholm, 1967 (ed J Östman) pp. 442-445. Excerpta Medica Foundation, Amsterdam 1969.
5. Dupré J J, Ross S. A., Watson D & Brown J. C. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J. clin. Endocr.* 37: 826, 1973.
6. Gennuth S. M. Metabolic clearance of insulin in man. *Diabetes* 1: 1003, 1972.
7. Goetz F. S., Manoy J. W. & Greenberg B. Z. The regulation of insulin secretion: Effects of the infusion of glucose, ribose and other sugars into the portal vein of dogs. *J. Lab. clin. Med.* 69: 537, 1967.
8. Iversen J. Effect of acetyl choline on the secretion of glucagon and insulin from the isolated perfused canine pancreas. *Diabetes* 22: 381, 1973.
9. Kaden M., Harding P. & Field J. B. Effect of intraduodenal glucose administration on hepatic extraction of insulin in anesthetized dog. *J. clin. Invest.* 52: 2016, 1973.
10. Kaplan N. & Madison L. L. Effects of endogenous insulin secretion on the magnitude of hepatic binding of labelled insulin during single transhepatic circulation in human subjects. *Clin. Res.* 7: 248, 1959.
11. Lund B., Aagaard P. & Deckert T. Effect of vagotomy on insulin release after oral and intravenous glucose administration. To be published.
12. Marliss E. B., Ginzler L., Seydoux J., Wolfheim C. B., Kanazawa Y., Orri L., Renold A. & Porte D. Glucagon release induced by pancreatic nerve stimulation in the dog. *J. clin. Invest.* 52: 1246, 1973.
13. McIntyre N., Holdsworth C. D. & Turner D. S. New interpretation of oral glucose tolerance. *Lancet* 2: 20, 1964.
14. McIntyre N., Turner D. S. & Holdsworth C. D. The role of the portal circulation in glucose and fructose tolerance. *Diabetologia* 8: 593, 1970.
15. Moody A. J., Marcussen J., Schachl Fries A., Steenstrup C., Sundby F., Malmose W. & Malmose-Lagae F. The insulin releasing activities of extracts of pork intestine. *Diabetologia* 6: 135, 1970.
16. Pork D., Ginzler L., Seydoux J., Kanazawa Y. & Posternak J. Neural regulation of insulin secretion in the dog. *J. clin. Invest.* 52: 210, 1973.
17. Raptis S., Dollinger H. C., Schröder K. E., Schleyer M., Rothschneider H. & Pfeiffer E. F. Differences in insulin, growth hormone and pancreatic enzyme secretion after intravenous and intraduodenal administration of mixed amino acids in man. *New Engl. J. Med.* 288: 1199, 1973.
18. Rehfeld J. F. & Studil F. The effect of gastric basal- and glucose-stimulated insulin secretion in man. *J. clin. Invest.* 52: 1415, 1973.
19. Rudeman N. & Herrera M. G. Glucose repara-

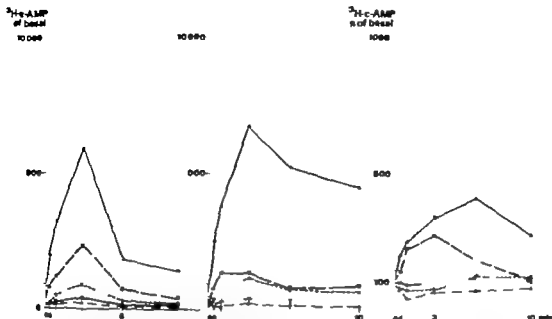


Fig. 2. Effects of noradrenaline (2×10^{-6} M), phenolamine ($5 \mu\text{g/ml}$) and theophylline (10^{-4} M) on $[^3\text{H}]$ cyclic AMP accumulation during 0.5–10 min of incubation. Results from 3 individual experiments are expressed as % of $[^3\text{H}]$ cyclic AMP in samples incubated under basal conditions.

Δ =noradrenaline \square =noradrenaline and phenolamine \triangle =noradrenaline, phenolamine and theophylline \blacksquare =noradrenaline and theophylline \circ =theophylline. Each point represents the mean of duplicates. Note the two different scales on the ordinate.

Short-term (30 sec) incubations

Effects of noradrenaline The effects of noradrenaline on $[^3\text{H}]$ cyclic AMP accumulation during 30 sec of incubation were tested. A very small stimulatory effect was observed when concentrations of 0.2×10^{-6} M and 2×10^{-6} M were used (Fig. 3) while 0.002×10^{-6} M and 0.02×10^{-6} M were without effect (data not shown).

Effects of phenolamine The addition of phenolamine (0.005 – $5 \mu\text{g/ml}$) potentiated the accumulation of $[^3\text{H}]$ cyclic AMP induced by noradrenaline the stimulation being more marked with increasing concentrations of the catecholamine (Figs. 3 and 5). Phenolamine per se tended to slightly depress basal $[^3\text{H}]$ cyclic AMP levels (Fig. 3).

Effects of propranolol The stimulation of $[^3\text{H}]$ cyclic AMP accumulation induced by $\times 10^{-6}$ M noradrenaline alone or in the presence of $5 \mu\text{g/ml}$ phenolamine was inhibited by 0.10 – $10 \mu\text{g/ml}$ propranolol, a total inhibition being obtained at concentrations of $1 \mu\text{g/ml}$ and above (Fig. 4).

Effects of theophylline The addition of 10^{-4} – 10^{-3} M theophylline significantly stimulated the accumulation of $[^3\text{H}]$ cyclic AMP and magnified the

stimulatory action of other agents (Figs. 3 and 5). The effect of 10^{-3} M theophylline alone was not significantly modified by the addition of phenolamine, $5 \mu\text{g/ml}$ ($-9.0 \pm 12.0\%$ mean \pm S.E.M. of difference in 11 experiments).

DISCUSSION

The present experiments have demonstrated rapid initial stimulation of $[^3\text{H}]$ cyclic AMP peak levels being reached within 3–6 min followed by declining levels of the nucleotide (Fig. 2). This pattern of response could not be markedly altered by theophylline, a known phosphodiesterase inhibitor nor by the α -adrenergic antagonist, phenolamine. These observations are in accordance with those of Manganiello et al. (16) and Ho and Sutherland (12) on fat cells from epididymal adipose tissue of rats. Ho and Sutherland presented data indicating that the decline or leveling off from peak cyclic AMP levels in adipose tissue was due to the generation and excretion of a hormone antagonist induced by elevated intracellular levels of cyclic AMP in the adipocyte. It is not known at present whether the above mentioned antagonistic substance decreases the

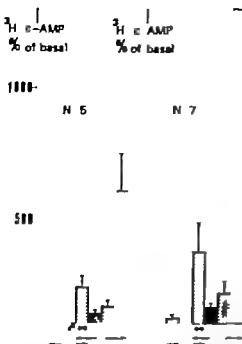


Fig. 3 Effect of noradrenaline, theophylline (1×10^{-3} M) and phentolamine ($5 \mu\text{g/ml}$) in 30 sec incubations. Noradrenaline 0.2×10^{-6} M (left) or 2.0×10^{-6} M (right) was added to incubations alone or together with theophylline or phentolamine. Columns from left to right in each part of the figure indicate incubations with noradrenaline, phentolamine, noradrenaline plus phentolamine, theophylline, noradrenaline plus theophylline and noradrenaline plus theophylline plus phentolamine, respectively. n = number of experiments (each performed with duplicate or triplicate incubations). Student t -test for paired differences was employed ($p < 0.05$, $p < 0.01$). P -values by arrows = comparisons between columns indicated by under columns; $+$ = significance of difference from values. Vertical bars = S.E.M.

synthesis or enhances the breakdown of cyclic AMP or both. Since one aim of the present study was to evaluate how synthesis and breakdown of cyclic AMP are affected by α -adrenergic receptors, the experiments were designed so as to minimize the influence of the above inhibitory mechanism. Since accumulation of $[^3\text{H}]$ cyclic AMP was shown to be approximately linear during the first 30 sec of incubation (Fig. 1) it was assumed that the adenyl cyclase activity is constant during this period. Therefore 30 sec incubations were used in the following experiments.

In order to evaluate whether the decrease in $[^3\text{H}]$ cyclic AMP induced by α -adrenergic stimulation was caused mainly by the stimulation of cyclic AMP breakdown, the effect of theophylline, a known

phosphodiesterase inhibitor was studied. It was assumed that if α -adrenergic receptor activity stimulated phosphodiesterase activity, theophylline would probably inhibit this effect. The validity of such an assumption is strengthened by the observation that theophylline can inhibit the stimulatory effects of other substances, e.g. cyclic GMP on phosphodiesterase enzymes (2). Our results show firstly that the effect of theophylline on $[^3\text{H}]$ cyclic AMP response to noradrenaline did not mimic the response to phentolamine ($5 \mu\text{g/ml}$) in the presence of noradrenaline (Fig. 3). Secondly, when different concentrations of phentolamine were used together

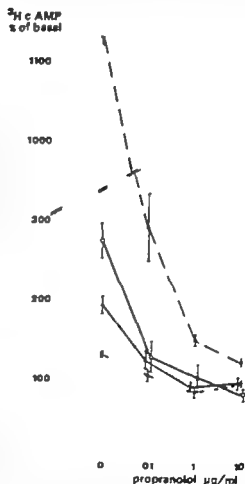


Fig. 4 Effects of propranolol (0.1 – $10 \mu\text{g/ml}$) on the $[^3\text{H}]$ cyclic AMP response induced by noradrenaline (2×10^{-6} M) in the presence or absence of phentolamine ($5 \mu\text{g/ml}$) and/or theophylline (1×10^{-3} M). Incubation time 30 sec. Results are expressed as % of basal values. Δ = noradrenaline plus phentolamine plus theophylline, O = noradrenaline plus phentolamine, \bullet = noradrenaline. Each point represents the mean \pm S.E.M. from 2 experiments.

^3H -cAMP
of basal

100

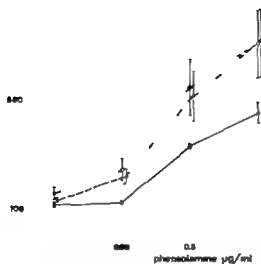


Fig. 5 Effects of phenolamine (0.05–5 µg/ml) on the ^3H cyclic AMP response to noradrenaline (0.2×10^{-6} M) in the presence or absence of theophylline (1×10^{-4} – 10^{-5} M) incubation time 30 sec. Results are given in % of basal values. — noradrenaline plus phenolamine —○— noradrenaline plus phenolamine plus theophylline (1×10^{-4} M) —△— noradrenaline plus phenolamine plus theophylline (1×10^{-5} M). Each point represents the mean \pm S.E.M. of three experiments.

with noradrenaline the concomitant addition of theophylline seemed to augment ^3H cyclic AMP to the same degree regardless of the presence of varying α -adrenergic blockade (Fig. 5). These results indicate that theophylline does not act as an α -adrenergic antagonist. Instead theophylline seems to have a multiplicative effect on the rate of ^3H cyclic AMP accumulation.

In conclusion our findings would seem to favour the hypothesis of α -adrenergic stimulation being mediated by inhibition of adenyl cyclase. It is realized however that an effect of α -adrenergic agents on cyclic AMP breakdown cannot wholly be excluded since theophylline does not block completely the phosphodiesterase enzymes.

ACKNOWLEDGEMENTS

This study was supported by grants from the Karolinska Institute, the Medical Research Committee of the Swedish Life Insurance Companies and the Karl Petrin Foundation.

REFERENCES

1. Abe, K., Robinson, G. A., Liddle, G. W., Butcher, R. W., Nicholson, W. E. & Baird, C. E. *Endocrinology* 85: 674 (1969).
2. Beavo, J. A., Rogers, N. L., Craford, O. B., Baird, C. E., Hardman, J. G., Sutherland, E. W. & Newman, E. V. *Ann. N.Y. Acad. Sci.* 185: 129 (1971).
3. Borris, T. W. & Langley, P. E. *J. Lab. clin. Med.* 75: 983 (1970).
4. Carlson, L. A. *Acta Soc. Med. Upsalen* 64: 208, 1959.
5. Chant, M., Rivkin, I., Marmak, F., Santoro, S. G. & Fleiss, S. *Int. J. Biol. Chem.* 246: 3037 (1971).
6. Doma, T. & Rychlik, I. *Biochem. biophys. Acta (Amst.)* 204: 1 (1970).
7. Elfvén, S. Studies on the effect of catecholamines on human adipose tissue metabolism. Thesis. Balder Stockholm 1970.
8. Ockerman, A. G. *Proc. nat. Acad. Sci. (Wash.)* 87: 305 (1970).
9. Goldrick, R. B. *Amer. J. Physiol.* 212: 777 (1967).
10. Grill, V. & Rosenqvist, U. *Acta med. scand.* 194: 129 (1973).
11. Handler, J., Benninger, R. & Orloff, J. *Amer. J. Physiol.* 215: 1024 (1968).
12. Ho, R. J. & Sutherland, E. W. *J. Biol. Chem.* 246: 6822, 1971.
13. Humes, J. L., Rombach, M. & Knebel, F. A. *Analyt. Biochem.* 32: 210, 1969.
14. Krishna, G., Weiss, B. & Brodie, B. B. *J. Pharmacol. exp. Ther.* 163: 379 (1968).
15. Kao, J. F. & DeRozas, E. C. *J. Biol. Chem.* 244: 2252, 1969.
16. Maroncello, V. C., Merad, F. & Vachon, M. *J. Biol. Chem.* 246: 2195 (1971).
17. Moskowitz, J., Harwood, J. P., Reed, W. III & Krishna, G. *Biochim. biophys. Acta (Amst.)* 230: 779 (1971).
18. Robison, G. A., Langley, P. E. & Burns, T. W. *Biochem. Pharmacol.* 1: 589 (1972).
19. Robison, G. A. & Sutherland, E. W. *Circulat. Res. Suppl.* 1: 1970.
20. Rodbell, M. *J. Biol. Chem.* 239: 371 (1964).
21. Smith, U. Experimental studies on human adipose tissue. With special reference to cell size. Thesis. Göttingen 1970.
22. Solomon, S. *J. Lab. clin. Med.* 79: 598, 1972.
23. Turtle, J. R. & Kupers, D. M. *Biochem. biophys. Res. Commun.* 28: 797 (1967).

REMOVAL OF EXOGENOUS TRIGLYCERIDES IN HUMAN FOREARM MUSCLE AND SUBCUTANEOUS TISSUE

Lennart Kaljser and Stephan Rössner

From King Gustaf V Research Institute and the Departments of Clinical Physiology and Internal Medicine Karolinska Hospital, Stockholm S-141 86

Abstract The removal of exogenous triglyceride (TG) in forearm muscle and subcutaneous tissue of 7 healthy male volunteers has been studied by nephelometric determinations of arterial-deep venous (a-dv) and arterial-superficial venous (a-sv) differences in concentration of fat particles. Exogenous TG was administered as constant i.v. infusion of Intralipid® over a period of 30 min at rest and another 15 min during forearm work. At rest a significant positive a-dv difference in fat particle concentration of $121 \pm 21 \mu\text{mol TG/l}$ (mean \pm S.E.M.) was found, which corresponds to a fractional extraction of $10.3 \pm 2.0\%$. Also the a-sv difference was significant $81 \pm 14 \mu\text{mol TG/l}$ $5.7 \pm 0.8\%$ of the arterial concentration. During exercise no further significant removal was found. Thus both skeletal muscle and subcutaneous tissue seem to be able to remove exogenous TG in substantial amounts under resting conditions. In the exercising muscle, however the direct removal of exogenous TG does not seem to be of significant importance.

Exogenous triglycerides (TG) both in the form of chylomicrons and as an artificial fat emulsion given intravenously are rapidly cleared from the blood. Chylomicron removal in the whole body has been studied in several species such as rat (9, 10) and dog (4, 20) and also in man (13). In general it follows zero order kinetics above a certain plasma concentration and first order kinetics below that level (4). In the lower concentration range 50% of isotopically labelled chylomicrons are removed from the blood after 5-12 min (9, 22). At this point of time after injection most of the label is found in the liver, adipose tissue and skeletal muscle but also in the heart a significant amount is found (2, 10). Whereas chylomicrons are believed to be removed intact by the liver hydrolysis by lipoprotein lipase seems to be a prerequisite for the extraction of chylomicron fatty

acids by adipose tissue, heart and skeletal muscle (21, 22).

Chylomicron TG are a rich potential source of energy. Of interest, then, is the extent to which under physiological conditions they are directly extracted and utilized by tissues with a high energy demand such as heart muscle and exercising skeletal muscle. The oxidative metabolism of the heart is under most conditions covered to an extent of about two-thirds by the oxidation of fatty acids (17). In studies where arterial-coronary sinus differences for chylomicrons were measured nephelometrically and at the same time TG differences chemically we could further show that substantial amounts of exogenous fat were extracted (5).

Skeletal muscle also utilizes fatty acids although these contribute less to oxidative metabolism than in heart muscle (12, 14). However the extent to which skeletal muscle can take up exogenous TG has not been studied in man under physiological conditions. The aim of this study was therefore to determine the possible extraction of exogenous TG in resting and exercising human skeletal muscle. The forearm musculature was chosen since it offers the possibility to sample blood from the deep venous system, which almost exclusively drains muscle tissue. The forearm also contains subcutaneous tissue. The subcutaneous tissue contains a large proportion of fat, which has a potential capacity of chylomicron removal. As it is possible also to sample blood from veins which predominantly drain these tissues the simultaneous removal of exogenous plasma TG in subcutaneous tissue was also studied. Exogenous TG was given to the subjects in the

Table II Arterial concentrations (a) and concentration differences of exogenous plasma TG ($\mu\text{mol/l}$) determined by nephelometry between artery and deep vein (a-dv) (means \pm S.E.M.)

Subj. no		a-dv	% of a		a-d	% of a
<i>Rest</i>	20 min			30 min		
1	1 622 \pm 15	327 \pm 20**	20.2	1 561 \pm 15	190 \pm 20***	12.1
2	906 \pm 6	173 \pm 10*	17.5	1 020 \pm 3	97 \pm 5**	9.5
3	1 241 \pm 5	109 \pm 8**	8.8	1 322 \pm 4	166 \pm 7***	12.6
4	1 487 \pm 12	30 \pm 13	2.0	1 678 \pm 4	84 \pm 12***	5.0
5	673 \pm 7	135 \pm 8***	21.2	643 \pm 5	140 \pm 6	21.8
6	1 881 \pm 13	60 \pm 20*	3.2	2 088 \pm 20	96 \pm 24**	4.6
7	1 390 \pm 11	20 \pm 15**	1.4	1 514 \pm 6	62 \pm 9***	4.1
$\bar{X} \pm$ S.E.M.		122 \pm 40	10.6 \pm 3.3		119 \pm 18***	10.0 \pm 2.4
<i>Exercise</i>	10 min			15 min		
1	1 635 \pm 19	5 \pm 40**	0.3	1 613 \pm 13	-66 \pm 15**	-4.1
2	1 088 \pm 3	13 \pm 6	1.3	1 012 \pm 12	33 \pm 8***	3.4
3	1 349 \pm 4	-16 \pm 6	-1.2	1 339 \pm 3	-57 \pm 6	-4.3
4	1 933 \pm 7	-91 \pm 13	-4.7	2 031 \pm 11	-57 \pm 18***	-2.8
5	635 \pm 7	18 \pm 8	2.9	630 \pm 5	31 \pm 8**	5.0
6	4 340 \pm 16	-62 \pm 25	-2.6	2 490 \pm 16	31 \pm 25**	1.4
7	1 622 \pm 7	-33 \pm 13	-2.0	1 639 \pm 5	58 \pm 8***	-3.5
$\bar{X} \pm$ S.E.M.		-23 \pm 15**	-0.9 \pm 1.0**		-20 \pm 19**	-0.7 \pm 1.3**

=not significant, $p < 0.05$ * $p < 0.01$ * $p < 0.001$

The statistical evaluation is based on the test of difference between means.

DISCUSSION

Methodology In this study the arterio-venous concentration differences of a fat emulsion have been determined by nephelometry which measures plasma turbidity. There are however several reasons to believe that such a method also measures quantitatively the exogenous plasma TG concentration difference across the vascular bed. In earlier studies comparisons

have been made between nephelometry and a chemical method and showed a high degree of agreement between the methods (5, 18). No systematic arteriovenous concentration differences of ^{125}I -albumin were found in some samples, however significant differences were observed and the arteriovenous differences of fat particles were corrected accordingly. However the results of the study are not influenced by these corrections.

Table III Arterial concentrations (a) and concentration differences of exogenous plasma TG ($\mu\text{mol/l}$) determined by nephelometry between artery and superficial vein (a-sv) (means \pm S.E.M.)

Subj. no		a	a-sv	% of a		a	a-sv	% of a
<i>Rest</i>	20 min				30 min			
1	1 622 \pm 15	202 \pm 10***	12.5		1 561 \pm 15	86 \pm 9***	5.5	
2	906 \pm 6	60 \pm 7*	6.0		1 020 \pm 3	80 \pm 7**	7.7	
3	1 241 \pm 5	45 \pm 9***	3.6		1 322 \pm 4	97 \pm 6**	7.3	
4	1 487 \pm 12	50 \pm 11	3.4		1 678 \pm 4	68 \pm 13	4.1	
5	673 \pm 7	93 \pm 6**	6.4		643 \pm 5	18 \pm 7**	3.0	
6	1 881 \pm 13	51 \pm 18**	2.8		2 088 \pm 20	127 \pm 22***	6.1	
7	1 390 \pm 11	-	-		1 514 \pm 6	-	-	
$\bar{X} \pm$ S.E.M.		84 \pm 25	5.8 \pm 1.5			79 \pm 15	5.6 \pm 0.7**	

Statistical symbols as in Table II.

Extraction of oxygen and lactate The oxygen extraction at rest in the forearm musculature and superficial tissue as well as the oxygen extraction and lactate release in the submaximally working forearm are in agreement with earlier data from healthy young subjects (15-24). The far lower a-v difference for oxygen in subcutaneous tissue than in muscle signifies a far greater blood flow in relation to oxidative metabolic rate—a fact of importance when the extraction of TG in the tissues is discussed.

Removal of exogenous plasma TG at rest At rest there was a significant removal of Intralipid® both at 20 and 30 min in the skeletal muscle of the forearm. This removal corresponded to about 10% of the arterial concentration of fat particles. During the same time the subcutaneous tissues of the forearm removed about 8% of the arterial concentration of fat particles. The smaller fractional removal in subcutaneous tissue might be due to the greater blood flow in this tissue in relation to its metabolic demand. Similar extraction figures for the splanchnic region have recently been found in a parallel study in which an arterio-portal extraction of 5.3% of the arterial concentration of fat particles was demonstrated during the same period (11). In an earlier study on myocardial removal of exogenous plasma TG the corresponding removal figure was 6% although this figure was the mean obtained from samplings taken at 1, 2, 3 and 4 hours during an infusion of Intralipid®. From the present short-term study it is not possible to conclude whether the skeletal muscle removal represents a steady uptake which can be maintained for a longer time.

Removal of exogenous plasma TG during work. No mean arteriovenous concentration differences of fat particles was observed during work. An explanation of this finding might be that the maximal rate of removal is utilized already at rest, with a resulting marked decrease in a-v concentration difference when the forearm blood flow is increased during work, presumably about five times (24). Support for the conception that skeletal muscle cannot increase its uptake of exogenous TG during work is found in a study in which Intralipid® was given as a constant rate i.v. infusion over 4 hours and the steady arterial concentration of exogenous TG was not influenced when the subjects started moderately heavy bicycle work after 2 hours (6). However, an alterna-

tive explanation might be that the fat particles removed are not taken up by the muscle tissue but merely adhered to the vessel walls. At the onset of forearm work they might then be washed out again maybe by the mechanical effect of the muscle contractions. The finding of some both positive and negative significant a-d concentration differences during work might to a certain extent support the interpretation that the fat particles are not taken up but loosely attached to the vessel walls.

ACKNOWLEDGEMENTS

Supported by grants from Vitrum AB, Stockholm, and the Swedish Medical Research Council (19X 204).

REFERENCES

- Block, W. D., Jarrett, K. J. & Leolne B. Use of single color reagent to improve the automated determination of serum total cholesterol. In: *Automation in analytical chemistry* vol. 1 (ed. L. T. Skogg) p. 345. Mediad, New York 1963.
- Braddon, J. M. & Gordon, R. S. Jr. Tissue distribution of C^{14} after the intravenous injection of labelled chylomicrons and monoterified fatty acid in the rat. *J. clin. Invest.* 37: 574 1958.
- Carlson, L. A. Determination of serum triglycerides. *J. Atheroscler. Res.* 3: 334 1963.
- Carlson, L. A. & Hallberg, D. Studies on the elimination of exogenous lipids from the blood stream. The kinetics of the elimination of fat emulsion and of chylomicrons in the dog after single injection. *Acta physiol. scand.* 59: 52, 1963.
- Carlson, L. A., Karper, L., Rösner, S. & Wahlqvist, M. L. Myocardial metabolism of exogenous plasma triglycerides in man. Studies during alimentary lipaemia and intravenous infusion of fat emulsion. *Acta med. scand.* 193: 233 1973.
- Myocardial metabolism during infusions of glucose and fat emulsion in healthy men: studies at rest and during prolonged exercise in preparation.
- Carlson, L. A. & Rösner, S. A methodological study of an intravenous fat tolerance test with Intralipid® emulsion. *Scand. J. clin. Lab. Invest.* 29: 243 1972.
- Coles, D. R., Cooper, K. E., Mottram, R. F. & Occlshaw, J. V. The source of blood samples withdrawn from deep forearm veins via catheters passed upstream from the median cubital vein. *J. Physiol. (Lond.)* 142: 323 1958.
- French, J. E. & Morris, B. The removal of ^{14}C -labelled chylomicron fat from the circulation in rats. *J. Physiol. (Lond.)* 138: 326, 1957.
- The tissue distribution and oxidation of ^{14}C -labelled chylomicron fat injected intravenously in rats. *J. Physiol. (Lond.)* 140: 261, 1958.

- 11 Freyachuss, U. Hallberg, D. Johnson L. & Rössner S. Removal of exogenous plasma, triglycerides in splanchnic viscera in man during anaesthesia. *Acta med scand* 196, 415 1974
- 12 Hagenfeldt, L. & Wahren, J. Human forearm muscle metabolism during exercise II. Uptake release and oxidation of individual FFA and glycerol. *Scand. J. clin. Lab. Invest.* 21 263 1968.
- 13 Hallberg, D. Studies on the elimination of exogenous lipids from the blood stream. Determination and separation of the plasma triglycerides after single injection of a fat emulsion in man. *Acta physiol. scand* 62, 407 1964
- 14 Havel, R. J. Pernow B. & Jones N. L. Uptake and release of free fatty acids and other metabolites in the legs of exercising man. *J. appl. Physiol.* 23 90 1967
- 15 Kaijser L. Limiting factors for aerobic muscle performance. *Acta physiol. scand. Suppl.* 346 1970
- 16 Kessler G. & Lederer H. Fluorimetric measurement of triglycerides. In *Automation in analytical chemistry* vol 1 (ed L. T. Skeggs), p. 341. McGraw-Hill New York 1965
- 17 Lessner, B. W. Kaijser L. & Carlson, L. A. Myocardial lipid and carbohydrate metabolism in healthy fasting men at rest studies during continuous infusion of ^3H -palmitate. *Europ. J. clin. Invest.* 2, 348 1972.
- 18 Lewis, B. Boberg, J. Mancini M. & Carlson, L. A. Determination of the intravenous fat tolerance test with Intralipid® by nephelometry. *Atherosclerosis* 15 83 1972.
- 19 Lundholm L. Möhne-Lundholm E. & Vamso N. Lactic acid assay with L. (+) lactic acid dehydrogenase from rabbit muscle. *Acta physiol. scand* 58 243 1963
- 20 Nestel P. J. Havel, R. J. & Bezman, A. Sites of initial removal of chylomicron triglyceride fatty acids from the blood. *J. clin. Invest.* 41 1915 1962.
- 21 Olivecrona, T. Metabolism of chylomicrons labelled with ^{14}C -glycerol- ^3H -palmitic acid in the rat. *J. Lipid Res.* 3 1 1962
- 22 Olivecrona, T. & Beffrage, P. Mechanisms for removal of chyle triglyceride from the circulating blood as studied with (^{14}C) glycerol and (^3H) palmitic acid-labelled chyle. *Biochim. biophys. Acta (Amst.)* 98: 81 1965
- 23 Scherrer M. König, J. & Möslé P. Vergleich der mit dem IL-CO Oximeter - Model 182 und der nach van Slyke ermittelten O_2 -Sättigung des Blutes. Einfluss von Methämoglobin und anderen Farbstoffen. *Schw. med. Wochr.* 101 1399 1971
- 24 Wahren, J. Quantitative aspects of blood flow and oxygen uptake in the human forearm during rhythmic exercise. *Acta physiol. scand. Suppl.* 269 1966.

Table 1 Plasma triglycerides (TG) and cholesterol (CHOL), body fat (BF), fat cell size (FCS) and k -value for intravenous glucose tolerance (IVGTT) and per cent of linoleic (18:2), linolenic (18:3) and arachidonic (20:4) acid in the extracted adipose tissue lipids in male subjects with different types of hyperlipoproteinemia

	Types of hyperlipoproteinemia					
	N	II A	II B	III	IV	V
n	14	3	2	4	18	2
TG (mmol/l)	1.46±0.47	1.65±0.63	3.86±2.09	5.75±4.58	4.37±1.96	13.43±4.98
CHOL (mg/100 ml)	220±37	351±29	317±27	358±137	272±33	533±49
BF (kg)	11.9±5.7	9.1±1.6	8.5±0.3	14.9±4.2	18.1±4.9**	16.4±0.1
FCS (μ)	75±13	84±4	85±18	87±15	88±5	85±1
IVGTT (k -value)	1.86±0.83	1.63±0.74	0.72±0.48	1.06±0.24	1.21±0.57*	1.10±0.01
18:2	11.77±4.47	11.78±1.42	13.90±0.60	13.17±3.62	11.10±1.12	10.22±0.31
18:3	1.30±0.78	1.97±0.77	1.06±0.07	1.70±0.64	1.32±0.47**	1.08±0.17*
20:4	0.97±0.50	0.60±0.38	0.08±0.12	0.40±0.30*	0.37±0.22*	0.23±0.02

According to the WHO classification, N=normal.

** indicate that the difference in relation to group III was significant with a p -value of 0.05, 0.01 and 0.001 (Student's t -test).

than normals. The content of 18:3 and 20:4 was low in the groups with HTG, while that of 18:2 was the same in all groups. On the other hand subjects with hypercholesterolemia but normal plasma TG levels did not differ from the normals.

The correlation coefficients between the metabolic and morphologic parameters are given in Table II. The content of 18:3 and 20:4 correlated positively to the k value but negatively to plasma TG levels. Body fat and fat cell size were not significantly correlated to the k value but negatively to the content of 18:3 and 20:4.

As there may be multiple interactions between all these parameters, the relationships have been further analysed by partial correlation analysis. Table III reveals that the correlation coefficient between the k value and 18:3 is only slightly changed from 0.59 to 0.51 when plasma TG are kept constant, and that between plasma TG and 18:3

goes from -0.55 to -0.44 when the k -value is kept constant. When the influence not only of plasma TG but also of fat cell size and body fat was eliminated the correlation between the k value and 18:3 was still approximately the same (Table III).

When the association between the k value and the TG concentration was analysed by calculation of partial correlation coefficients (Table IV), it was found that this association remained when fat cell size or body fat was kept constant but disappeared when 18:3 was kept constant.

DISCUSSION

The results thus show that with decreasing percentage of 18:3 in AT the intravenous glucose tolerance declined and the plasma triglyceride concentration increased. These two associations with

Table II Correlation coefficients between plasma TG, the k -value for the IVGTT and different adipose tissue characteristics

Abbreviations, see Table I

	BF	μ	log TG	18:3	20:4
log k	-0.17	-0.13	-0.36	0.59***	0.47*
BF		0.59*	0.53***	-0.45	-0.46**
μ			0.49***	-0.43**	-0.54**
log TG				-0.55**	-0.55***
18:3					0.85***
20:4					

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table III Relation between 18:3 and the k value for the IVGTT and plasma TG concentration (partial correlation coefficients)

Abbreviations, see Table I

Parameters correlated	Parameters kept constant	r -value
$\log k$ 18:3	$\log TG$.51
$\log TG$ 18:3	$\log k$	-.44
$\log k$ 18:3	$\log TG$ FCS BF	.54

18:3 were largely independent of each other. The usual relation between GI and HTG (2) however appeared to be due to their common association with the content of 18:3. This suggests that the high frequency of GI found in HTG might be related to a low content of 18:3 in AT associated with both HTG and GI. It has indeed been suggested that the association between GI and HTG may be mediated through a common association to the same factor, perhaps adiposity (2). Since the correlation between plasma TG and the k value was not diminished appreciably when partial correlation was performed and body fat was kept constant, our results rather suggest that the common factor is AT 18:3 which in fact was related to adiposity (Table II).

The present relations were found for the percentage of 18:3 in AT. Since there was no significant relation between the k value and body fat or fat cell size, and since the correlation coefficient was unaffected when the influence of body fat was eliminated, it is unlikely that the relation between the k -value and 18:3 was due to changes in body fat.

Similar relations to those for 18:3 were also found for 20:4. It should be stressed that these acids have only been identified tentatively by retention time factors.

In earlier studies some abnormalities have been found in the fatty acid composition of various plasma lipid fractions in different types of hyperlipidemia or in atherosclerosis, the most common finding being a decrease in 18:2. Schmelde et al. (10) thus observed a negative correlation between the percentage of 18:2 and 20:4 and total plasma lipids. Similarly Kingsbury et al. (8) found lower 18:2 and 20:4 in subcutaneous AT in subjects with atherosclerotic diseases than in a control material. It is difficult to connect these findings with our finding of normal 18:2 in AT. Hagenfeldt et al. (7) recently reported a low content of 18:3 and 20:4 in the plasma free fatty acids (FFA) in patients having survived a myocardial infarction. Since such patients frequently have either HTG or GI, it is possible that their AT content of 18:3 and 20:4 was low, which might cause a low FFA content of these acids since the majority of plasma FFA are mobilized from AT.

Both 18:3 and 20:4 can be derived from 18:2, while 18:2 cannot be synthesized in the body but must be provided in the diet. Since the content of 18:2 in AT was normal, it seems likely that the low content of 18:3 and 20:4 is due rather to changed metabolism than to a dietary deficiency. Of interest in this connection is the finding in diabetic animals that there is an impaired synthesis of arachidonic acid from linoleic acid (6).

ACKNOWLEDGEMENT

Supported by grants from the Swedish Medical Research Foundation (19X/704) and Svenska Marzbrandstörns Näringsfysiologiska Forsning.

REFERENCES

1. Beaumont, J. L., Carlson, L. A., Cooper, G. R., Pfeiffer, Z., Fredrickson, D. S. & Strasser, T. *Bull. Wild. Hlth. Org.* 43: 381, 1970.
2. Bierman, E. L., Porte, D. & Bagdade, J. D. Jr. In: *Adipose tissue: Regulation and metabolic functions* (ed. J. Jeannerod and D. Hepp), p. 209. Academic Press, London, 1970.
3. Björntorp, M., Gustafsson, A. & Pernow, B. *Acta med. scand.* 190: 363, 1971.
4. Björntorp, M. & Östman, J. *Ad. metabol. Disord.* 5: 277, 1971.
5. Dole, V. P. *J. clin. Invest.* 35: 150, 1956.
6. Friedmann, N., Gellhorn, A. & Bergman, W. *Israel J. med. Sci.* 2: 677, 1966.

Table IV Relation between the k value for the IVGTT and plasma TG concentration (partial correlation coefficients)

Abbreviations, see Table I

Parameters correlated	Parameters kept constant	r -value
k $\log TG$		-.36
k $\log TG$	FCS	-.35
k $\log TG$	BF	-.32
k $\log TG$	18:3	-.05

- 7 Hagenfeldt L, Paasikivi J & Sjögren A. *Metabolism* 22: 1349 1973
- 8 Kingsbury K. J. Morgan D. M. Aylott C. Burton, P. Emerson, R. & Robinson, P. J. *Clin. Sci* 33: 161 1962.
- 9 Persson, B. *Acta med scand* 193: 447 1973
- 10 Schnade, W., Biegler R. & Böhm E.. *J Atheroscler Res.* 1: 47 1961
- 11 Sjöström, L., Björntorp P. & Vrana, J.. *J Lipid Res.* 12: 521 1971
- 12 Walldius, G. & Rubba, P. In manuscript 1974

POLYMORPHIC ACETYLATION OF PROCAINE AMIDE IN HEALTHY SUBJECTS

Erling Karlsson and Lillian Molin

From the Departments of Clinical Pharmacology and Internal Medicine, Division of Cardiology, University of Linköping, Linköping, and the Department of Clinical Pharmacology at Karolinska Institute, Huddinge Hospital, Huddinge, Sweden

Abstract The acetylation of procaine amide has been studied by means of gas liquid chromatography in 33 healthy human volunteers. The acetylator phenotype was determined by measuring unchanged and acetylated sulphapyridine in urine. Slow acetylators of sulphapyridine excreted significantly less procaine amide in acetylated form in the urine than rapid acetylators ($9 \pm 1\%$ against $19 \pm 4\%$). Hence it is suggested that the acetylation of procaine amide is subject to the same genetic polymorphism as that of isoniazid and some sulfonamides.

Procaine amide has been shown to be an effective drug in the treatment of ventricular arrhythmias (7, 8, 12) but has the disadvantage of inducing a systemic lupus erythematosus (SLE)-like syndrome in some patients during long-term treatment (1).

A similar syndrome can also be induced by hydralazine which like isoniazid and some sulfonamides has been shown to be polymorphically acetylated in man (6). Since this SLE-like syndrome occurs predominantly in the phenotype slow acetylators (14) it was of interest to explore the possibility that also procaine amide is acetylated polymorphically in man. Indirect evidence for this had already been obtained by investigating the biotransformation of procaine amide in cardiac patients (9, 10). However these patients were not drug-free and some of them also had renal impairment, which complicated interpretation of the results. Therefore we thought it important to study procaine amide acetylation in healthy subjects who on a separate occasion underwent a sulphapyridine phenotyping test.

MATERIAL AND METHODS

Thirty-three healthy and drug-free volunteers, 6 females and 27 males, 23-46 years old (mean 26), participated in the

study. Their state of health was determined by history, physical examination, ECG and routine laboratory tests including Hb, ESR, serum creatinine and serum levels of bilirubin, alkaline phosphatases, GOT, OPT and LDH activities. All subjects gave their informed consent.

The subjects were first phenotyped as slow or fast acetylators of sulphapyridine in the following way. The fasting subjects were given 500 mg sulphapyridine (as commercial Septipalmon tablets supplied by AB Pharmacia, Uppsala, Sweden) in the morning and food intake was not allowed until at least 1 hour later. Urine specimens from the 7th-8th hour after the oral intake of sulphapyridine were collected and analysed within 1 day according to Schröder and Evans (16).

At least 1 week later the procedure was repeated with procaine amide. The fasting subjects were given 500 mg procaine amide hydrochloride orally as the commercial tablets of 250 mg (Procenyl® Squibb). One blood sample was drawn just before the dose and used as plasma blank. The other blood samples were drawn 1, 2.5, 5 and 7 hours after the dose in order to verify adequate absorption of the drug. The concentration of procaine amide in plasma was determined by the spectrofluorometric method described by Koch-Weser and Klei (11) but substituting benzene for toluene. Plasma samples were stored at -20°C for up to 1 month before analysis.

Urine was collected for 8 hours at 4 intervals (0-1, 1-4, 4-6, and 6-8 hours after drug ingestion). Urine samples were stored at $+4^{\circ}\text{C}$ for up to 2 weeks before analysis of procaine amide and N-acetylprocaine amide.

The method used for analysis of procaine amide and N-acetylprocaine amide has been described in detail in previous paper (10). Because of the lower concentration predicted in this investigation compared to that in cardiac patients, standard urine samples containing 80, 250, 500 and 1600 $\mu\text{g/ml}$ procaine amide and 20, 60, 160, and 320 $\mu\text{g/ml}$ N-acetylprocaine amide were analysed in every assay procedure for specimens from volunteer subjects. It was found to be also suitable to inject 10 μl sample into the gas chromatograph for the same reason. A 5% OV17 chromatographic column prepared in the same manner as the column used for plasma analysis in our earlier study was used for urinary samples. All determinations were performed in duplicate together with urinary blanks.

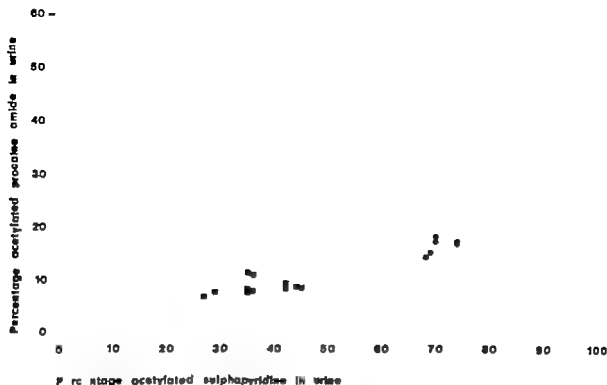


Fig. 1 Relationship between percentage of acetylated sulphapyridine in urine and urinary N-acetylprocaine amide in % of the sum of excreted procaine amide and

N-acetylprocaine amide. Rapid (●) and slow (■) acetylators according to sulphapyridine test ($n=33$)

Statistical methods

The significance of differences between means was tested by Student's *t*-test. The significance of correlations between acetylated sulphapyridine and procaine amide was using Spearman's rank correlation (17).

RESULTS

Rapid and slow acetylator phenotypes were clearly defined by means of the sulphapyridine test (Fig. 1). The mean percentage of acetylated sulphapyridine in the urine of slow acetylators was $37 \pm 6\%$ (mean \pm S.D.) and in the urine of rapid acetylators $72 \pm 4\%$ (mean \pm S.D.). Seventeen of 33 subjects (51.5%) were slow acetylators which is within the same range as reported by Schröder and Evans (16) when investigating volunteers.

In fast acetylators of sulphapyridine the urinary excretion of N-acetylprocaine amide accounted for $19 \pm 4\%$ (mean \pm S.D.) of the total sum of recovered procaine amide and N-acetylprocaine amide in the urinary specimen collected during 1–4 hours after administered dose. The corresponding percentage in the slow acetylator group was $9 \pm 1\%$ (mean \pm S.D.)

The differences between the group means reached statistical significance ($p < 0.001$).

A significant correlation was found between the percentage acetylation of sulphapyridine and procaine amide in urine ($r=0.80$; $p < 0.001$, Spearman's rank correlation). Table 1 gives the urinary excretion of acetylated procaine amide in all the different urine portions for slow and rapid acetylators. In a frequency distribution histogram the best separation of groups was obtained with the data from the urine collected 1–4 hours after administered dose.

Table 1 Percentage acetylation of procaine amide in the different urine portions with comparison between slow ($n=17$) and rapid ($n=16$) acetylator phenotypes determined by means of sulphapyridine (mean \pm S.D.)

Hours after procaine amide ingestion	Slow acetylators	Rapid acetylators
0–1	4.0 ± 1.0	8.2 ± 2.0
1–4	8.7 ± 1.3	18.9 ± 4.0
4–6	14.9 ± 2.2	31.2 ± 6.1
6–8	21.8 ± 3.2	41.5 ± 7.6
0–8	10.6 ± 1.6	22.5 ± 4.7

Table II Plasma concentration and plasma half life of procaine amide in slow and fast acetylators (mean \pm S.D.)

	Hours after dose	Plasma concentration of procaine amide (μ g/ml)	Plasma half life of procaine amide (min)
Fast ($n=16$)	1	1.2 ± 0.5	176 \pm 33
	2.5	1.6 ± 0.2	
	5	0.8 ± 0.2	
	7	0.5 ± 0.1	
Slow ($n=17$)	1	2.3 ± 0.4	180 \pm 38
	2.5	1.5 ± 0.5	
	5	0.8 ± 0.3	
	7	0.6 ± 0.1	

The mean plasma concentration of procaine amide did not differ between slow and fast sulphapyridine acetylators nor did the plasma half-life of procaine amide (Table II).

DISCUSSION

The incidence of slow acetylators in our material was about 50% which is within the range reported by Schröder and Evans (16). Conclusive evidence has been presented that the acetylation of procaine amide follows the same pattern as that of sulphapyridine. There is a significant intrasubject correlation between the acetylation rate of the two drugs showing that procaine amide can be added to the list of drugs whose acetylation is governed by the same genetic polymorphism.

There was no difference between rapid and slow acetylators regarding either the initial (or mean) plasma concentration or the plasma half-life of unchanged procaine amide. This is probably explained by the fact that procaine amide is excreted unchanged in the urine to a great extent. It has recently been suggested that hydralazine is acetylated during the first passage through the intestine or liver explaining the lower peak levels but identical half-lives of the drug in fast than in slow acetylators (2, 15). Evidently this mechanism does not play a major role for procaine amide.

N-acetylprocaine amide is a potentially active metabolite (4, 5, 9) that accumulates in renal failure. Preliminary data show that the relation between N-acetylprocaine amide and unchanged procaine amide in plasma is 3:2 in patients with impaired renal function whilst in patients with normal kidney

function the ratio is the reverse (10). The degree of accumulation of N-acetylprocaine amide can be expected to differ between the two phenotypes in renal failure. Phenotyping such patients may thus be of clinical value.

Among the drugs which have been proven to induce an SLE-like syndrome procaine amide, hydralazine and isoniazid seem to feature most frequently in published reports (13). Slow acetylators have been shown to be more prone than rapid ones to develop various side-effects after the latter two drugs e.g. polyneuritis after isoniazid (3) and the SLE-like syndrome after hydralazine (14). It will therefore be of considerable interest to determine whether a similar relationship exists for procaine amide too. Further studies with determination of the phenotype in patients who have developed a procaine amide-induced SLE-like syndrome are needed to answer this question.

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish National Association against Heart and Chest Diseases.

REFERENCES

- Blomgren, S. E., Condemi, J. J. & Vaughan, J. H. Procaineamide-induced lupus erythematosus: Clinical and laboratory observations. *Am J Med* 52: 338, 1972.
- Collier, P., Frick-Holmberg, M., Gerle, M., Rawlins, M., Sjöqvist, P. & Östman, J. Antihypertensiva medels övervak. III. Hydralazin och kloralidon. Medicinsk Riksstämman, Stockholm 1973.
- Davadas, S., Gangadhara, P. R. J., Andrews, R. H., Fox, W., Ramakrishnan, C. V., Sefton, J. B. & Velu, S. Peripheral neuritis due to isoniazid. *Bull. Wild Hlth Org* 23: 587, 1960.
- Drayer, D. E., Reidenberg, M. M. & Sevy, R. W. N-acetylprocaineamide: An active metabolite of procaineamide (38104). *Proc. Soc. exp. Biol. (N. Y.)* 146: 358, 1974.
- Elson, J., Strong, J. M. & Adanson, A. J. Jr. Antiarhythmic potency of N-acetylprocaineamide. *Clin Pharmacol Ther* 15: 204, 1974.
- Evans, D. A. P. & Whittle, T. A. Human acetylation polymorphism. *J. Lab. clin. Med.* 63: 394, 1964.
- García, E.-G. V., Hansenbottel, R. H. & Bigler, J. Th., Jr. Intravenous intravenous procaine amide to treat ventricular arrhythmias. Correlation of plasma concentration with effect on arrhythmias, electrocardiogram and blood pressure. *Ann. Intern. Med.* 78: 183, 1973.
- Karlsson, E. Procaine amide and phenytoin. A comparative study of their antiarrhythmic effects at apparent therapeutic plasma levels. To be published.

- 9 Karlsson E, Aberg G, Collste, P, Molin, L, Norlander B & Sjöqvist, F. Acetylation of procaine amide in man—a preliminary communication. *Europ J clin Pharmacol*. In press 1974
- 10 Karlsson E, Molin L, Norlander B & Sjöqvist F. Acetylation of procaine amide in man studied with a new gas chromatographic method. *Brit. J cl Pharmacol*. In press 1974
- 11 Koch-Weser J & Klein S W. Procainamide dosage schedules, plasma concentrations and clinical effects. *J A M A* 215 1454 1971
- 12 Koch-Weser J, Klein, S W, Foo-Canto L, L, Kastor J A. & DeSmetts, R. W. Antiarrhythmic prophylaxis with procainamide in acute myocardial infarction. *New Engl J Med* 281 1253 1969
- 13 Lee S L. & Siegel M. Drug-induced systemic lupus erythematosus. In: *Drug diseases* 3 || 239. *Excerpta med Fund Amsterdam* 1968
- 14 Perry H M Jr. Late toxicity to hydralazine resembling systemic lupus erythematosus or rheumatoid arthritis. *Amer J Med* 54 58 1973
- 15 Rikdénberg, M. M, Drayer D, DeMarco A. L., & Carmon T B. Hydralazine elimination in man. *Clin. Pharmacol Ther* 14 970 1973
- 16 Schröder H & Evans, D A. P. The polymorphic acetylation of sulphapyridine in man. *J med Genet.* 9-168, 1972.
- 17 Siegel S. *Nonparametric statistics for the behavioral sciences*. McGraw-Hill New York 1956.

ACETYLATOR PHENOTYPE AND THE ANTIHYPERTENSIVE RESPONSE TO HYDRALAZINE

A. J. Jounela, M. Pesanen and M. J. Mattila

From the Departments of Medicine, Maria Hospital, Helsinki, Kemi Central Hospital, Kemi, and the Department of Pharmacology, University of Helsinki, Helsinki, Finland

Abstract Twenty-three out-patients with mild or moderate essential hypertension have been treated with a combination of hydralazine (57.5-150 mg daily) and oxprenolol (60 mg daily). Before treatment the patients were phenotyped for polymorphic acetylation by means of the sulphamethazine test. 12 proved to be 'slow' and 11 'rapid' acetylators. A significant correlation was found between daily doses of hydralazine and the plasma hydralazine levels, separately in slow ($r=0.480$) and in rapid ($r=0.580$) acetylators. The antihypertensive response to hydralazine correlated well to plasma hydralazine levels. The mean fall of BP in slow acetylators was 33/23 mmHg in supine and 20/18 mmHg in standing position. The corresponding values in rapid acetylators were 22/15 and 21/13 mmHg. The average daily doses of hydralazine needed for these responses were 1.3 mg/kg in slow and 1.6 mg/kg in rapid acetylators. To reduce the systolic BP by 20 mmHg, 1.0 mg/kg of hydralazine was needed in slow acetylators, rapid acetylators needed significantly higher dose of 1.4 mg/kg. During a follow-up of 1 year there have been virtually no side-effects. The results tally with the previous findings of Zacest and Koch-Weser who demonstrated a similar correlation during the triple-drug regimen. It seems as if hypertensive patients can be successfully treated with hydralazine and β -blocking drugs without knowledge of the patient's acetylator phenotype. However, acetylator status is determinant of tissue levels and long-term toxicity of hydralazine and patients should be phenotyped because β -blockers may mask the warning side-effects.

Like isoniazid and sulphamethazine (SMZ) hydralazine (HZ) is acetylated on the polymorphic pattern (2). The polymorphism of acetylation correlates to the activity of liver enzyme acetyltransferase: the slow acetylator phenotype is an autosomal recessive trait and there are racial differences with regard to its frequency (2). Slow acetylators are liable to generate antinuclear antibodies or systemic lupus during long-term

treatment with HZ (9). This is probably due to an accumulation of HZ in tissues since Zacest and Koch-Weser (13) have shown that the same dose given to slow acetylators results in higher plasma HZ levels than in rapid acetylators.

It is known that the antihypertensive response to HZ in rats is related to its concentration in blood and aortic wall (6). We initiated the present trial to correlate the acetylator status and plasma HZ levels of hypertensive patients to their antihypertensive response to HZ. During our trial Zacest and Koch-Weser (13) reported that this correlation exists in a situation where HZ is administered in combination with propranolol and diuretics.

PATIENTS AND METHODS

Patients

Twenty-three out-patients, 12 males and 11 females, with established essential hypertension, were admitted to the trial. Their ages ranged from 24 to 58 years (mean 46) and their weights from 61 to 118 kg (mean 80). The patients were accepted for the trial if their diastolic BP measured during three subsequent visits in the Out-patient Clinic at 1-week intervals, was consistently 95 mmHg or more. All cases with history of angina pectoris, cardiac decompensation, asthma or astolomene diseases were excluded. After fulfilling these criteria the following tests were obtained in all patients: complete blood count, ESR, serum electrolytes, creatinine and uric acid, urinalysis, 24-hour urinary excretion of metanephrines, LE cells, rheumatoid factor and antinuclear antibodies, ECG, chest X-ray, pyelography and examination of ocular fundi. On these grounds the patients were classified to fulfil the criteria of grades I-II hypertension (WHO).

Treatment of blood pressure

BP was measured throughout the study in the Out-patient Clinic by the same trained

the same standard mercury sphygmomanometer. Three to six measurements at 5-min intervals in supine and 2-3 measurements in standing position were taken between 4 and 6 p.m. and the three lowest values were averaged. Heart rate was counted from the radial pulse after the last BP recording in each position. Patients were checked in the Out-patient Clinic at weekly intervals. At each visit they were questioned about side-effects and their general condition, BP and heart rate were measured and tablets were counted. At the end of the trial, which lasted 3 months and one year after starting the treatment, blood count, ESR, urinalysis, serum creatinine, LE cells and antinuclear antibodies were investigated. After the three visits during which the baseline BP was recorded, treatment was started with HZ at a dose of 12.5 mg three times daily.

Since our previous experience showed that treatment with HZ alone up to 150 mg daily resulted in therapeutic failure and intolerable side-effects, the treatment with a β -adrenoceptor blocking drug oxprenolol (20 mg t.i.d.) was combined with the HZ treatment from the very beginning. The dose of HZ was increased at 1-week intervals by 37.5 mg up to a total daily dose of 150 mg or until the patients were normotensive or until intolerable side-effects occurred.

Chemical measurement of drugs

The polymorphic acetylator phenotype of the patients was determined by using SMZ as test drug since it is acetylated like isoniazid and HZ (2) and easy to measure. SMZ tablets (10 mg/kg) were given to the patients during the first visit to the Out-patient Clinic. In the morning of the day of their second visit they took a sample of morning urine for a reference, ingested the tablets with a light breakfast, and the 6-hour urine sample was taken in the Out-patient Clinic. The aliquots of urine were stored at

-20°C for several weeks before SMZ and acetyl SMZ were assayed by the chemical Bratton-Marshall procedure (12). This simple measurement of unacetylated and acetylated SMZ in urine is accurate enough to separate slow and rapid acetylators (7).

To estimate the plasma HZ, samples of venous blood were drawn during the visits 2-4 hours following the last dose of HZ. Plasma was separated by centrifugation, acidified by adding two drops of 10 N hydrochloric acid, and kept at -20°C for several weeks until assayed according to the spectrophotometric method (11) which in our hands proved reliable in the concentration range of 0.06-4 μ g/ml.

Statistical analysis

Student's *t* test was used for the statistical analysis of the results.

RESULTS

Acetylator phenotype and plasma HZ levels On the basis of present results and our previous experience (3) 12 patients of a total of 23 were classified as slow acetylators, their ratios of unacetylated SMZ/total SMZ in urine ranging from 29 to 52%. The respective ratios in 11 rapid acetylators ranged from 6 to 19%.

The plasma HZ levels related to the HZ dosage are illustrated in Fig. 1. Generally the plasma levels were higher with increasing doses but the highest levels were not reached in rapid acetylators. When the plasma HZ levels are plotted against the HZ dosage separately in either phenotype the correla-

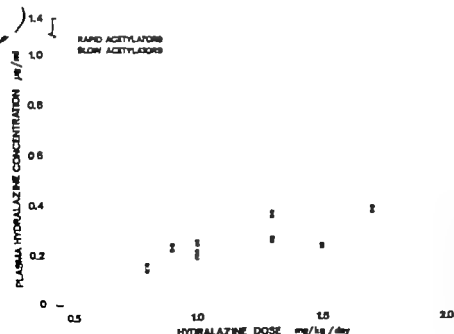


Fig. 1 Correlation between daily hydralazine dose and plasma level of the drug in slow ($r=0.480$) and in rapid ($r=0.580$) acetylators.

Table 1 Effect of combined hydralazine+oxprenolol on blood pressure (mmHg: mean \pm S.E.) in slow and rapid acetylators

	Supine BP		Standing BP	
	Systolic	Diastolic	Systolic	Diastolic
<i>Slow acetylators</i>				
Before treatment	176 \pm 6	114 \pm 4	164 \pm 7	115 \pm 4
During treatment	141 \pm 3	91 \pm 2	144 \pm 5	97 \pm 3
<i>Rapid acetylators</i>				
Before treatment	167 \pm 5	110 \pm 1	166 \pm 8	112 \pm 2
During treatment	145 \pm 4	95 \pm 3	145 \pm 5	97 \pm 3

tions are statistically significant ($p < 0.05$) in both instances.

Since the homozygous or heterozygous acetylators genotype can be revealed by special technique, and the amount of polymorphic liver acetyl transferase is consequently known to be higher in homozygous than in heterozygous rapid acetylators, the plasma HZ levels achieved within a narrow dose range 1.2–1.6 mg/kg were plotted against the SMZ/acetylation ratio unacetylated SMZ/total SMZ. It appeared that high HZ levels ($>0.5 \mu\text{g/ml}$) were measured in slow acetylators with an SMZ ratio above 35% whereas all

rapid acetylators and some slow acetylators exhibited lower HZ levels.

Antihypertensive response to HZ The dose of oxprenolol used (20 mg i.i.d.) which is far below its actual antihypertensive dose range (4) proved effective in preventing the tachycardia and headache that are associated with moderate doses of HZ alone. The antihypertensive response of the patients to treatment is seen in Table 1. There was evidently a satisfactory clinical effect yet the patients remained on the borderline hypertensive level. To obtain these responses in slow acetylators an average HZ dose of 1.3 mg/kg was needed compared with 1.6 mg/kg in rapid acetylators. The difference is not significant.

The average reduction of supine diastolic pressure in slow acetylators (23 mmHg) was significantly more ($p < 0.05$) than in rapid acetylators (15 mmHg). This indicates that slow acetylators were slightly more responsive to given dose of HZ. In order to reduce supine systolic BP by 20 mmHg or more, 1.0 mg/kg HZ was needed in slow acetylators and 1.4 mg/kg in rapid. This difference is statistically significant ($p < 0.05$).

Fig. 2 illustrates the correlation between the antihypertensive response and plasma HZ levels, with the data for slow and rapid acetylators combined. The response improves with increasing

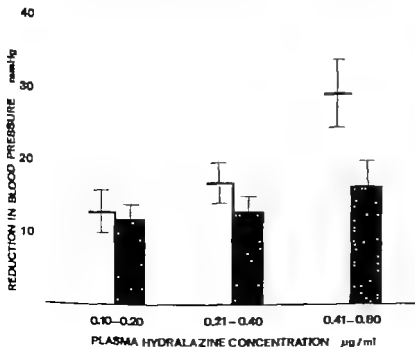


Fig. 2 Effect of plasma hydralazine concentration on the antihypertensive response in all patients studied. Open columns=systolic, stippled columns=diastolic BP. Mean \pm S.E.

Table III 24-hour urinary excretion of trace elements (μg) with no known biological function before and during treatment with hydralazine

Case no	Ag		As		Au		Cs	
	Before	During	Before	During	Before	During	Before	During
1	0.46	0.32	42	210	0.00055		14	11
	0.43	1.6	610	510	0.0021	0.0044	10	13
3	0.37	0.52	100	67	0.066	0.019	15	17
4	0.41	0.58	85	66	0.00072		8.9	18
5	0.47	0.28	110	86	0.00087	0.00087		7.0
Mean \pm S.D.	0.43 \pm 0.04	0.66 \pm 0.54	190 \pm 240	190 \pm 190	0.014 \pm 0.029	0.0081 \pm 0.0096	12 \pm 3	13 \pm 8
Mean difference	+0.23		-2.2		-0.016		+2.0	

that hydralazine disease may be related to a metal deficiency (2, 10).

It has been reported that hypertensive patients excrete large amounts of cadmium and manganese in the urine and that treatment with hydralazine lowers the urinary excretion of these elements (6). However in a previous study I found that the urinary excretion of 17 trace elements in untreated hypertensive patients did not differ significantly from that in normotensive subjects (11).

The aim of the present study was to investigate the urinary excretion of antimony, arsenic, bromine, cadmium, cesium, cobalt, copper, gold, iron, mercury, molybdenum, rubidium, scandium, selenium, silver, tungsten and zinc in hypertensive patients before and during treatment with hydralazine.

MATERIAL AND METHODS

Five male hypertensive patients, ranging in age from 29 to 56 years, were subjected to the study. Three patients had mild hypertension with diastolic BP on admission varying between 105 and 115 mmHg. The other two patients (nos. 3 and 5) had more severe hypertension with diastolic BP on admission of 150 and 125 mmHg, respectively. Patient 2 also had an enlargement of the heart (870 cm³/m²) and this patient and one of the others (no. 5) had slight proteinuria. The patients had not previously received any antihypertensive medication. They were treated with hydralazine (Apresol[®] 75 mg/day) and five 4-hour urine samples were pooled and 1 ml sample of the pooled urine was pipetted into quartz ampoules for trace element analysis. In a previous study (11) urine from untreated patients was analysed in the same manner.

The trace element determinations were performed with neutron activation analysis. The neutron flux used was $2 \cdot 10^{12}$ n/cm²/sec and the samples, together with stand-

ards, were irradiated for 4 hours. The chemical separation technique has been described elsewhere (7, 12).

Statistical methods

The statistical analysis was undertaken with the paired *t*-test. The degrees of significance have been expressed as follows: not significant, $0.05 < p$; almost significant, $0.01 < p < 0.05$; significant, $0.001 < p < 0.01$; highly significant, $p < 0.001$.

RESULTS

The urinary excretion of the different trace elements studied before and during treatment with hydralazine is presented in Tables I-III. Mean values, S.D. and paired mean differences have been calculated. The amounts excreted are given in $\mu\text{g}/24$ hours. In these Tables the trace elements have been divided into those with known (Table I) suspected (Table II) and no known biological function (Table III).

Among the trace elements with known biological function, it was noted that the mean amount of copper excreted during treatment with hydralazine was more than twice that before treatment; the paired mean difference of 89 $\mu\text{g}/24$ hours was almost significant. On the other hand the urinary excretion of zinc was almost significantly lower during treatment compared to before. The excretion of cobalt and iron did not change significantly.

The paired mean differences for all five trace elements with suspected biological function were very small and insignificant among the trace elements with no known biological function no significant differences were observed between the urinary excretion during and before treatment. The results expressed in $\mu\text{g/g}$ excreted creatinine per 4 hours gave the same statistical correlations.

Hg		Sb		Sc		W	
Before	During	Before	During	Before	During	Before	During
0.66	0.45	1.7	2.0	0.070	0.22	41	5.6
1.8	1.3	2.4	1.9	0.034	0.014	12	5.6
2.4		2.4	1.4	0.016	0.038	16	16
2.4	2.7	1.3	2.0		0.021	6.5	4.9
1.4	1.9	2.0	0.67	0.0078	0.0064	3.0	1.3
1.7±0.73	1.6±0.95	2.0±0.57	1.6±0.57	0.029±0.028	0.060±0.090	16±15	6.3±5.7
-0.002		-0.41		+0.014		-9.4	

DISCUSSION

The most prominent finding in this study is the increased urinary excretion of copper during treatment with hydralazine. The mean value before treatment (77 µg/24 h) is somewhat higher than the mean found previously (50 µg/24 h) in a larger group of untreated hypertensive patients (11). This is probably due to the fact that two of the patients in the present study had slight proteinuria, since copper excretion is elevated in this disorder (1-4). However the increase in this excretion during treatment with hydralazine cannot be explained by proteinuria as three of the patients did not have this disorder and in the two who did the proteinuria did not increase during the treatment.

It has also been reported that zinc excretion is increased in patients with proteinuria (3) which explains the somewhat higher mean value for the untreated patients in the present study as compared to that obtained previously in a larger group of untreated hypertensive patients (11).

The clinical significance of the increased urinary copper excretion during treatment with hydralazine is difficult to interpret. It is not known whether the increase is a temporary phenomenon or whether it persists during treatment for longer periods, neither has the possibility been studied that copper concentrations decrease in serum or tissues.

ACKNOWLEDGEMENT

This investigation was supported by the Swedish National Association Against Heart and Chest Diseases and by Karolinska Institute.

REFERENCES

- Adelstein S J & Vallee B L. Copper metabolism in man. *New Engl J Med* 265: 892, 1961.
- Comert P. Chronic intoxication from hydralazine resembling disseminated lupus erythematosus and apparent reversal by manganous I. Metal-binding in medicine p 312. Lippincott, Philadelphia 1960.
- McCamre, R. A. & Widdowson E. M. The absorption and excretion of zinc. *Biochem J* 36: 692, 1942.
- Mench-Petersen S. On the copper content in urine in proteinuria. *Biochemical Inst. Århus University Denmark* 1960.
- Perry H. M. & Schroeder H. A. Studies on the control of hypertension by kypale. III. Pharmacological and chemical observations on 1-hydrazinophthalazine. *Amer J Med Sci* 228: 396, 1954.
- Concentration of trace metals in urine of treated and untreated hypertensive patients compared with normal subjects. *J Lab. clin Med* 46: 936, 1955.
- Samsaál, K. Weiser P. O. & Lundström O. A automatic group separation system for the simultaneous determination of great number of elements in biological material. Recovery and reproducibility studies. *Analyt Chem* 40: 181, 1968.
- Schroeder H. A. Trace metal and chronic diseases. *Advanc Intern Med* 8: 59, 1954.
- Mechanisms of hypertension. Thomas Springfield 1957.
- Possible relationships between trace metals and chronic diseases. In: Metal-binding in medicine, p 99. Lippincott, Philadelphia 1960.
- Wester H. O. Trace elements in serum and urine from hypertensive patients before and during treatment with chlorothalidone. *Acta med scand* 194: 504, 1973.
- Wester P. O. Bruce H. & Samsaál, K. Radiochemical recovery studies of separation scheme for 33 elements in biological material. *Int J appl Radiat* 15: 39, 1964.

FEVER AND HAEMOLYSIS IN HODGKIN'S DISEASE

L. Storgaard and H. Karle

From Department of Medicine A, Division of Haematology, Rigshospitalet
University Hospital, Copenhagen, Denmark

Abstract. One hundred and four patients with Hodgkin disease have been studied retrospectively in order to evaluate the relationship between fever associated with the disease (Pel-Ebstein type) and the development of anaemia. In the material 19 episodes of fever were found to fit this type. The mean loss of Hb during fever period was 14% (range 3-33). From the rate of decrease in Hb it was deduced that this was at least partly caused by an increased destruction of erythrocytes. There was a significant correlation between the thermal exposure (expressed either as the duration of fever, the maximum body temperature during the fever period, or the sum of the temperature maxima) and the degree of erythrocyte loss. The loss of Hb was self-limited in spite of persistent fever. Furthermore, there seemed to be an inverse relationship between the degree of preexisting anaemia and the fever-induced relative loss of Hb. A possible explanation is that the older part of the erythrocyte population is more sensitive to the effect of fever.

Haemolytic episodes in conjunction with fever of the Pel-Ebstein type in Hodgkin's disease have been described in a few cases (4-11). From investigations in rabbits it has been suggested that elevation of the body temperature within the fever range induces an increased destruction of erythrocytes (5-7). Furthermore, *in vitro* studies have shown that human erythrocytes are also sensitive to destruction by small rises in temperature (8-9).

The aim of the present study was to examine a possible general relationship between the elevation of body temperature during Pel-Ebstein fever and erythrocyte destruction in patients with Hodgkin disease.

MATERIAL AND METHODS

Altogether 104 cases of Hodgkin disease confirmed by biopsy admitted to the department in the period 1969-73 were studied retrospectively. In 30 of the patients febrile episodes were observed. 9 patients were excluded from this material because of difficulties in interpreting changes

in the Hb level (bleeding, transfusions, cytotoxic treatment). The remaining 1 patients had a total of 26 episodes of fever. In 19 of these episodes there were no signs of infection as judged by clinical examination, bacteriological studies of blood and urine, and chest X-ray and consequently the fever was considered to be related to the disease itself. In most cases the fever curve was of the Pel-Ebstein type. In 6 cases the febrile episode was caused by pneumonia and in 1 case by acute cystitis.

Eighteen of the patients were in stages III or IV and one patient in stage II (Paris-Liège classification). One patient had splenomegaly, two had hepatomegaly and in one patient splenectomy had been performed years before.

Only elevations in temperature above 37.5°C for more than one day were registered, and all calculations were based upon the maximum value for each day of fever. The degree of heat exposure was expressed as 1) the duration of the fever period, 2) the highest temperature measured during the fever period, or 3) the sum of the daily maximum values throughout the fever period (fever index).

The haematological consequences of an attack of fever were estimated from changes in the Hb concentration and the reticulocyte counts. The changes in Hb were expressed as the loss (%) during the fever period. Major bleeding was excluded on clinical grounds. In all cases the direct Coombs test was negative.

RESULTS

During the 19 episodes of fever the mean decrease in the Hb concentration was 14% (range 3-33). In order to examine the mechanism of the decrease in Hb the results from the early part of the fever period were analysed in detail. The mean decrease during the first week of fever was 11% with an average of 1.7°C/day (range 0.6-3.2). During the same period the mean reticulocyte count was 0.9% (range 0.4-2.0).

Fig. 1 shows the relationship between the duration of fever and the loss of Hb as a percentage of the prefebrile Hb concentration. Although the results have a rather wide scatter, regression analysis

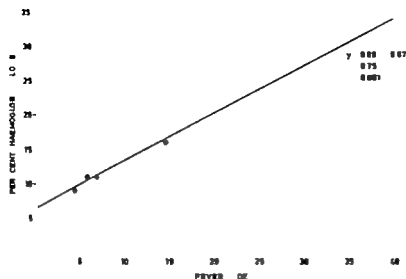


Fig 3 Relationship between fever index and loss of Hb

plained by a decreasing effect on the RBC with increasing fever index.

The results in the patients with hepatosplenomegaly and the patient who previously had a splenectomy did not differ significantly from the other part of the material.

In Fig. 4 the loss of Hb is related to the prefebrile Hb concentration in each fever period. The patients are divided into two groups according to their max

imum fever level. In the low temperature group ($<40^{\circ}\text{C}$) there was a significant increase ($p < 0.05$) in the loss of Hb with increasing values of prefebrile Hb. Also in the high temperature group ($>40^{\circ}\text{C}$) such a correlation seems to exist. As seen in Fig. 4 the curve is steeper although the result is not significant ($p > 0.1$). Both regression lines intersect with the abscissa in the region of about 6 g/100 ml, thus indicating a lower limit of prefebrile Hb below

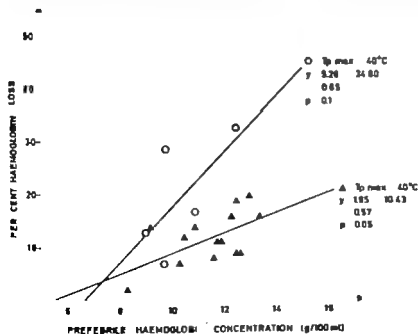


Fig 4 Relationship between the prefebrile Hb conc and the relative loss of Hb during fever. The patients are divided into two groups according to the temperature maximum.

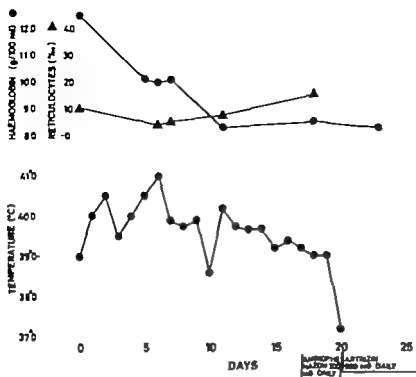


Fig. 5 Course of Hb concentration and reticulocyte count in relation to a fever episode in one of the patients.

which no decrease in the Hb concentration occurs as a result of fever.

Fig. 5 shows the changes in the Hb concentration during the course of a fever period and again the importance of the Hb level for the degree of erythrocyte destruction is illustrated. During the initial of the fever period (about 10 days) there is a rise in Hb from 12.5 to about 8.3 g/100 ml. After a rise in spite of continuous fever for more than 1 week, the Hb concentration seems to be constant. The stabilization of the Hb level does not seem to be due to an increase in erythropoiesis as there was no increase in the reticulocyte count. Also using the rate of decline in Hb concentration during the first 10 days of fever as a parameter of the intensity of haemolytic activity it can be calculated that the level of stabilization in the later part of the fever period (provided haemolytic activity is unchanged) should have been lower if all the red cells were equally sensitive.

Full restitution of the Hb concentration was not seen for several days after the fever had subsided indicating that the changes in Hb concentration could be only partly due to changes in the plasma volume during the fever period.

The influence of any erythropoietic response on the changes in the Hb concentration was estimated

from the reticulocyte count during the fever period. There was no sign of increase in the reticulocyte count (mean $\pm 0.5\%$ range 0.3–1.7).

DISCUSSION

This study shows a general tendency towards a decrease in Hb concentration in conjunction with episodes of fever in patients with Hodgkin's disease. From the rate of decrease in Hb (1.7%/day) compared with the reticulocyte count in the initial part of the fever period it follows that this is due to increased erythrocyte destruction since major bleeding or changes in plasma volume could be excluded.

The present study gives no direct information about the mechanism of the erythrocyte destruction, and several possibilities should be considered. In this series the direct Coombs test was negative but this does not entirely exclude an autoimmune mechanism as discussed by Ranlev and Videbæk (11).

Another explanation could be that erythrocyte survival depends in some way directly on the body temperature. The quantitative estimate of the thermal exposure used in this study based either on the duration of fever, the maximum temperature meas-

ured during the fever period, or the sum of the temperature maximum, represents an obvious approximation. Both the absolute rise in body temperature and the duration of the exposure may be important but the same fever index can be obtained from either a short intensive fever period or a prolonged fever period with only a slight increase in temperature. Furthermore, if there is a maximum susceptibility of the red cell population above which no further destruction occurs any attempt to correlate the erythrocyte destruction with the exposure expressed as either the duration of fever or the fever index will give a misleading result. Nevertheless the investigation demonstrates a correlation between the degree of thermal exposure and the loss of Hb.

Experimental studies in rabbits using different methods of increasing body temperature (5-7) as well as *in vitro* studies (8-9) support the hypothesis that temperatures within the fever range increase the destruction of red cells. The findings of the present study may be a clinical correlate to those results.

Fig. 5 shows that a maximum erythrocyte destruction is obtained in spite of persistent fever. This pattern of self-limited cell loss which was not explained by a compensatory increase in erythropoiesis, is in accordance with previous findings during experimental fever in rabbits (7). Also, the influence of the prefebrile Hb level on the relative loss during fever (Fig. 4) points to a self-limited type of erythrocyte destruction. A possible explanation is that only the older part of the erythrocyte population is susceptible to the heat exposure, as has been shown for rabbit erythrocytes (6).

To elucidate this problem different models of erythrocyte destruction have been outlined in Fig. 6. Each part of the figure represents the cell age profile of the erythrocyte population by showing the fraction of cells at various cell ages. In both column A and B three levels of total prefebrile erythrocyte mass are demonstrated viz E (normal state) $3/4 E$ and $1/2 E$. Column A demonstrates situations with an unchanged cell age profile at different degrees of prefebrile anaemia and column B situations in which prefebrile anaemia is accompanied by a change in the cell age profile with decreasing number of older cells. The heat sensitive erythrocytes are symbolized by the hatched area, and, using a simplification it is assumed that all erythrocytes above a certain cell age will be destroyed by

fever. Irrespective of the mechanism in relation to cell age the situation in column A cannot explain the results seen in this study as this would give a constant relative cell loss at different levels of prefebrile Hb and no lower threshold as indicated in Fig. 4. Only the situation presented in column B including a skew cell age distribution and the hypothesis of heat-induced erythrocyte destruction as a cell age dependent function, will explain a decreasing relative Hb loss with increasing prefebrile anaemia. The delineation of the cell age profile in relation to anaemia in Hodgkin's disease as in column B is in accordance with general concepts of erythrokinetics in malignancy (10) especially in Hodgkin's disease (3).

In accordance with the assumption of a self-limited process, the effect of fever is not very marked showing a mean Hb loss of 14% and a maximum loss of 33%. In this context it is interesting that the anaemia seen in a number of diseases with different aetiology and with fever as a central clinical feature, such as chronic infection, collagen disease, and malignancy is never very pronounced. The mechanism of this type of anaemia, for which the term anaemia of chronic disorders is generally used has never been clarified (11-12).

The result of fever has little practical implication

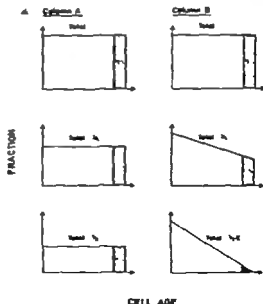


Fig. 6 Model constructed in an attempt to explain the influence of the prefebrile Hb level on fever-induced erythrocyte destruction in Hodgkin disease.

since it is rarely necessary to use blood transfusions to correct the anaemia seen in relation to fever. This moreover might be of limited value due to the transfusion of more erythrocytes sensitive to the effect of fever. An attempt to maintain a normal Hb level especially with old blood during fever will require disproportionately many transfusions and hence increase the risk of complications.

REFERENCES

- 1 Cartwright, G. E. The anaemia of chronic disorders. *Semin. Hematol.* 3:351 1966.
- 2 Cartwright, G. E. & Lee, G. R. The anaemia of chronic disorders. Annotation. *Brit. J. Haematol.* 21:147 1971.
- 3 Cline, M. J. & Berlin, N. I. Anaemia in Hodgkin disease. *Cancer* 16:526 1963.
- 4 Dacie, J. V. The haemolytic anaemias. Congenital and acquired. vol. 3, p. 722. Churchill, London 1967.
- 5 Karle, H.. Elevated body temperature and the survival of red blood cells. A study of experimental pyrexia in rabbits. *Acta med. scand.* 183:587 1968.
- 6 — Significance of red cell age to red cell destruction during experimental pyrexia. *Brit. J. Haematol.* 15:221 1968.
- 7 — Destruction of erythrocytes during experimental fever. Quantitative aspects. *Acta med. scand.* 186:349 1969.
- 8 — Effect on red cells of a small rise in temperature: *in vitro* studies. *Brit. J. Haematol.* 11:409 1969.
- 9 Karle, H. & Hansen, W. E. Changes in the red cell membrane induced by small rise in temperature. *Scand. J. clin. Lab. Invest.* 26:169 1970.
- 10 Price, V. E. & Greenfield, R. E. Anaemia in cancer. *Advances in cancer research*, p. 199. Academic Press, New York 1958.
- 11 Rønlov, P. & Videbeck, Aa. Cyclic haemolytic anaemia synchronous with Pel-Ebstein fever in a case of Hodgkin's disease. *Acta med. scand.* 174:93 1963.

THERAPEUTIC EFFECT OF LEO 1031 AN ALKYLATING CORTICOSTEROID ESTER IN LYMPHOPROLIFERATIVE DISORDERS

1 Chronic Lymphocytic Leukaemia

L. Brandt, I. Könyves and T. R. Möller

From the Departments of Internal Medicine and Radiotherapy, University Hospital, Lund
and the Research Laboratories AB Leo, Helsingborg, Sweden

Abstract. Leo 1031, a chlorambucil ester of prednisolone, has been administered orally in 15 patients with chronic lymphocytic leukaemia (CLL) continuously for 1-29 months (mean 12.5). Seven patients were previously untreated and eight had been treated with prednisolone, radiotherapy and/or alkylating agents. The initial daily dose was generally 8-16 mg and the maintenance dose was 6-8 mg. Allopurinol was given concurrently in 14 of 15 patients. Reduction of the leucocyte count was observed and a reduction, in most instances of lymphadenopathy or splenomegaly or both. In seven patients the Hb concentration was improved. Significant toxic effects on bone marrow function have been observed in one patient. Two patients developed proctitis. Our study suggests that the drug is effective in the treatment of CLL.

The use of a chemical combination of hormonal and alkylating agents seems logical in the treatment of several neoplastic disorders. These two types of drugs act differently within a given cell and may also affect different types of cells within a tumour mass (1).

With this as a starting point, a series of corticoid-alkylating agents was prepared by the Research Laboratories AB Leo, Helsingborg, Sweden. The synthesis of substances of this type has been reported by Könyves and Kristensson (2) and the relation between chemical structure and cytostatic activity by Könyves et al (3). Among these compounds the pregna-1,4-diene-3,20-dione 11,17,21-trihydroxy-21,4-p-(bis-(2-chloroethyl)-amino)phenylbutyrate (Leo 1031, EORTC 1502, NSC 134087, CB 400-037, p INN prednisusthine) was selected for further studies. The structural formula is presented in Fig. 1. Similar com-

pounds have been described (2, 9, 12) but no corticosteroid-cytostatic compound of the same type as Leo 1031 has been reported.

Because this new type of ester is lipophilic and differs markedly in physicochemical properties from the parent alkylating acid, it might be expected that the drug remains unaltered for a sufficient length of time to reach tumour sites that are inaccessible to the lipophobic free acid (5). The main reason for employing a corticosteroid as a carrier is to obtain a selective distribution, by localizing the alkylating agent in higher concentration within the malignant cells and in this way reduce the systemic toxicity. Such specific high-affinity binding of corticosteroids has been demonstrated, e.g. in lymphoma and CLL lymphocytes (6).

The effect of Leo 1031 on nine animal tumours has been described. The therapeutic indices (T.I.) were higher than those of chlorambucil in the majority of cases because of the lower toxicity of Leo 1031. The T.I. of Leo 1031 in solid Wistar 256 carcinoma is about twice that of the equivalent mixture of prednisolone and chlorambucil (10). Evenaar et al (3) found that Leo 1031 caused a volume reduction of transplantable osteosarcoma of the same magnitude as observed with nitrosoureas.

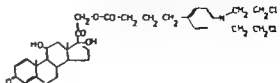


Fig. 1. Structural formula of Leo 1031.

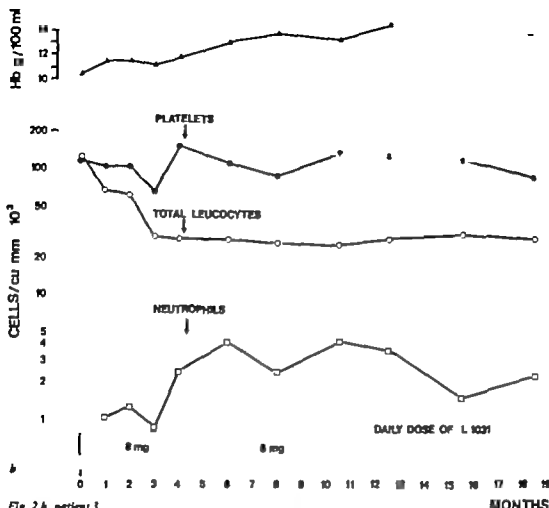


Fig 2b patient 3

though in one patient (no 8) the Hb later fell to initial level. Transient thrombocytopenia was in patient 10 concomitantly with urticaria after 8 weeks treatment (see Side-effects). Granulocytopenia was not observed in any patient. Two patients (nos 11 and 12) had previously received localized radiation therapy. Patient 11 has had marked symptoms of chronic bronchitis and emphysema for about 30 years. Treatment with Leo 1031 caused a reduction of leucocytosis, but the mild anaemia did not improve. Thrombocytopenia or granulocytopenia was not induced. Patient 12 had dermatomyositis complicating her haematologic disease. She had only a moderate leucocytosis which was not significantly reduced during treatment with Leo 1031. There was an increase in Hb concentration and no effects on the thrombocyte or granulocyte counts were observed. The symptoms elicited by dermatomyositis did not improve during the follow-up period.

Three patients (nos 13-15) had been treated previously with other alkylating agents. Patient 13 was administered chlorambucil 6 mg daily for 2 weeks but the drug was withdrawn owing to thrombocytopenia. This patient was also given prednisolone without improvement of the haematologic data. During treatment with Leo 1031 for 6 months a reduction of the leucocyte count was noted. There was however no improvement in her anaemia, and the thrombocyte and granulocyte counts became extremely low. This patient had a severe splenomegaly and splenectomy was performed. After splenectomy the Hb concentration rose and the thrombocyte and granulocyte counts were substantially improved during continuous administration of Leo 1031. This may indicate that the initial deterioration as reflected by the haematologic data, during treatment with the drug was probably not due to a toxic effect. Patient 14 had undergone treatment with cyclophosphamide

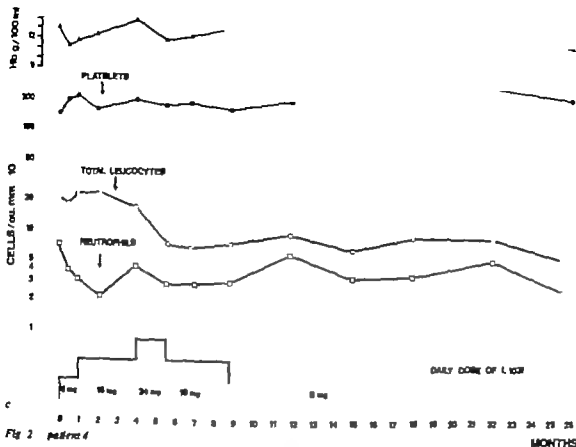


Fig 2 patient 4

MONTHS

for 40 months and had an increasing leucocytosis a falling Hb concentration and decreasing thrombocyte counts. During treatment with Leo 1031 there was a decreased leucocytosis. Thrombocytopenia persisted and the Hb concentration was successively reduced. The patient eventually succumbed with severe anaemia and septicaemia. Patient 15—who previously had been prescribed chlorambucil, 8 mg daily for a short period without effect on her anaemia—did not improve during treatment with Leo 1031 although the leucocyte count was reduced.

Side-effects

In two patients (nos. 7 and 10) urticaria and fever developed after 3 and 8 weeks' treatment, respectively. In patient 7 anaemia was aggravated during the reaction and transfusions were given. Granulocytopenia, 800/ μ l was also noted in this patient, but it could not be determined with any certainty whether the number of granulocytes was reduced from the pretreatment value.

Thrombocytopenia was found prior to treatment and was not significantly aggravated during the period with fever and urticaria. In patient 10 a transient thrombocytopenia, 75 000/ μ l was associated with the skin reaction. It must be emphasized that both patients were receiving allopurinol concurrently with Leo 1031 and it is therefore uncertain whether the side-effects were due to Leo 1031 or allopurinol. The drugs were withdrawn and the further course has been uncomplicated in both patients. The Hb concentration in patient 7 has risen spontaneously to 11.4 g/100 ml during a follow-up period of 12 months after the skin reaction.

Patient 9 experienced severe pain in the left shoulder joint shortly after the treatment started. In spite of the concurrent treatment with allopurinol serum uric acid was elevated and a clinical diagnosis of gout was made. Following treatment with oxyphenbutazone for 3 days the pain was relieved and has not reoccurred during continuous treatment with Leo 1031.

Gastrointestinal symptoms have not been

ported by any patient. No clinical signs of adverse steroid effects e.g. oedema or increased tendency to bruising, have been observed.

DISCUSSION

There seems to be little evidence that treatment of CLL significantly prolongs survival (4) and the main purpose of the therapy is to control troublesome symptoms caused by the disease. Therefore a therapeutic regimen without serious side-effects is desirable. Animal experiments with Leo 1031 indicated a low toxicity of the substance combined with a high therapeutic index in various experimental tumours (3, 10) and it was therefore considered justified to test the effect of the drug in CLL.

Leo 1031 was found to cause a significant reduction of the number of circulating lymphocytes as well as a reduction of enlarged lymph nodes and splenomegaly. Signs of bone marrow toxicity have been essentially lacking and in several patients an improvement in the anaemia has been observed. The drug has probably not induced thrombocytopenia in any patient, although low platelet counts were noted in one patient in connection with an allergic reaction. Granulocytopenia has been observed in a few patients but it is uncertain whether this was due to treatment or to the disease per se. Because low granulocyte counts have been found to increase in spite of continuous administration of the drug, it is improbable that Leo 1031 has a significant granulopoiesis-inhibiting effect in the dose given to the CLL patients. This conclusion is supported because the patients exposed to the drug for prolonged periods did not tend to have lower granulocyte counts than those who received the drug for a short period. The low bone marrow toxicity was confirmed in a series of patients with lymphocytic lymphoma who had received higher doses of Leo 1031 (11).

Our preliminary data thus point to a favourable effect of the drug in controlling CLL. Since the disease characteristically runs a slow course our observation period is however still too short to permit evaluation of the long-term effect of treatment with the drug.

REFERENCES

1. Brulé G, Eckhardt, S. J., Hall T. C. & Winkler A., Drug therapy of cancer p. 59 WHO Geneva 1973.
2. Catsoulacos P. EARC Symp Budapest Proc p. 23 1972.
3. Evenaar A. H., Wros E. H. M. & van Patten L. M. Cell killing effectiveness of an alkylating steroid (Leo 1031). *Europ. J. Cancer* 9: 773 1973.
4. Hansen M. M. Chronic lymphocytic leukaemia. Clinical studies based on 189 cases followed for a long time. *Scand. J. Haemat.* Suppl. 18: 131 1973.
5. Johns D. G., Paraphar D., Chabner H. A., Wolfert, H. K. & Adamson R. H. Antineoplastic activity of lipid-soluble distyl esters of methotrexate. *Experientia* 29: 1104 1973.
6. Kornel L., On the effects and the mechanisms of action of corticosteroids in normal and neoplastic target tissues. Findings and hypotheses. *Acta endocr. Suppl.* 178 1973.
7. Könyves I., Fex H. & Högborg, B. Novel corticosteroid esters with alkylating properties. Communication at 8th International Congress of Chemotherapy Athens Abstract No. B 159 1973.
8. Könyves I. & Kristenson S., Novel corticosteroid esters with alkylating properties. Communication at XIV Scandinavian Congress of Chemistry Umeå Proc. p. 187 1971.
9. Larionov L. F., Sofina, Z. P., Lagova, N. D., Sakodinskaya E. N., Yagzhinskaya, V. P. & Varlam O. S. *Vop. Oncol.* 14 (11): 61 1968.
10. Leo 1031 Brochure 3rd ed. Leo Helsingborg March 1973.
11. Möller T. R., Brandt, L., Könyves, I. & Lindberg, L. O. Therapeutic effect of Leo 1031 as alkylating corticosteroid ester in lymphoproliferative disorders. II. Lymphocytic lymphoma. *Acta med. scand.* 191: 323 1975.
12. Wall M. E., Abernethy G. S., J. Carroll, F. L. & Taylor H. J. *J. med. Chem.* 1: 810, 1969.

THERAPEUTIC EFFECT OF LEO 1031 AN ALKYLATING CORTICOSTEROID ESTER IN LYMPHOPROLIFERATIVE DISORDERS

II *Lymphocytic Lymphoma*

T. R. Möller, L. Brandt, I. Könyves and L. G. Lindberg

*From the Departments of Radiotherapy, Internal Medicine and Cytodiagnoses,
University Hospital Lund and the Research Laboratories AB Leo
Helsingborg, Sweden*

Abstract. Leo 1031, a chlorambucil ester of prednisolone, has been administered to 20 patients with generalized lymphocytic lymphoma (LL) of various histologic types. The average daily dose was 40 mg orally; the treatment was given continuously for 1-17 months (mean 7). Complete remission was obtained in five patients and partial remission in ten. The best results were obtained in patients with nodular type of LL. Significant leucopenia was induced in two patients, no thrombocytopenia has occurred in any patient. In two patients, castigooid labraris developed after 8 and 4 months, respectively. Leo 1031 may be of value as the single drug in the treatment of some types of LL. A combination therapy with Leo 1031 and vincristine has been tried in 5 of these patients, in 2 as initial therapy and in 3 later in association with relapse. Further trial to assess the value of Leo 1031 as part of a combination schedule are desirable.

Leo 1031, a chlorambucil ester of prednisolone, was synthesized in 1969 in the Research Laboratories AB Leo Helsingborg, Sweden (1, 13) and was made available for clinical trial in 1971 (14). In previously published reports (2, 3) the effect of the compound was evaluated in patients with chronic lymphocytic leukaemia. The present report deals with the effect of Leo 1031 in lymphocytic lymphoma (LL).

Previous investigations indicate that poor results can be expected from the treatment of LL ("lymphosarcoma") when employing single drug chemotherapy. Thus complete remission rates of 10-20% have been reported with chlorambucil, cyclophosphamide or Vinca alkaloids alone (4, 8, 11). With combination therapy more encouraging

results have been obtained, with a complete remission rate of about 90% (9, 15, 16). These programmes, however, often require hospitalization for certain periods. Since Leo 1031 contains two of the components usually employed in these programmes, i.e. alkylating agent and prednisolone, we considered it justifiable to investigate whether the effect of Leo 1031 in LL might be comparable to results reported with more elaborate programmes.

MATERIAL AND METHODS

Patients

Treatment with Leo 1031 was started in 23 patients with LL. Three patients were excluded from this study for various reasons: one patient (no. 4) turned to homeopathy after 2 weeks' treatment with Leo 1031; one (no. 5) died 4 days after starting treatment, and in one (no. 18) the cytologic diagnosis was changed to histiocytic lymphoma. Of the 20 patients thus included in the study, 8 were women and 12 men, with an age range of 29-80 years (mean 60).

Indication for treatment was in all patients generalized disease, i.e. stages III and IV according to the Ann Arbor classification (5). The staging procedure included in all patients chest X-ray, scintigraphy of the liver and spleen, bone marrow biopsy and fine-needle aspiration biopsy of the liver, spleen, palpable lymph nodes and other sites suspected of involvement. In addition, lymphography and inferior cavography were performed in most patients, but staging laparotomy was not included in the programme. As a result of the staging procedure, 8 patients were considered to have stage III disease and 12 stage IV. In the latter group, involvement of the bone marrow was confirmed in 8 cases, of the gastrointestinal tract in 4, of the skin and subcutaneous tissue in 4, of the skeleton in one

patient, and two patients had malignant pleural and peritoneal effusions respectively.

All diagnoses were confirmed through examination of a gross biopsy specimen and the lesions were classified according to Rappaport *et al.* (17). Of the nodular patterns, two patients had a well differentiated type of LL (NLWD), two a poorly differentiated type (NLPD) and two a lymphoma of mixed lymphocytic-histiocytic type (NM). Of the diffuse pattern, two patients had a well differentiated LL (DLWD), 11 poorly differentiated lymphocytic lymphoma (DLPD) and one a mixed lymphocytic-histiocytic type of lymphoma (DM). This distribution of the different types of lymphoma deviates from that usually reported (10) and may reflect non-intentional selection of patients.

Leo 1031 was given as the first therapeutic measure to 11 patients. Nine patients had been treated previously: viz. five with irradiation of localized disease, one with alkylating agents and three with both these methods.

Blood counts were performed in all patients biweekly or monthly during the treatment. Leucopenia was defined as a WBC count below 3000/ μ l and thrombocytopenia as a platelet count below 100 000/ μ l.

Administration of the drug

The drug was available in tablets containing 8 or 20 mg. At the beginning of the study the dose was kept rather low 4-24 mg daily (patients 1-5). The dose level for the remainder have varied between 32 and 80 mg daily, 40 mg being the most common dosage. Treatment was given continuously and even in the patients in whom a complete remission was obtained, maintenance therapy was given with the same dosage.

Table 1 Result of treatment with Leo 1031

Type of LL	No. of pts.	Remission		
		Complete	Partial	None
<i>Nodular</i>				
NLWD	2	1	1	
NLPD				
NM			1	1
<i>Diffuse</i>				
DLWD	2		2	
DLPD	11	11	6	3
DM	1			1
Total	20	5	10	5

Five patients were given vincristine (Oncovin®) concurrently with Leo 1031 in two initially and in three later in the course of treatment in order to achieve reinduction of remission. In all except one a cyclic schedule was tried. Leo 1031 was given for 2 weeks in a dose of 40 mg/m² daily and vincristine was given in a dose of 1.2 mg/m² on days 1-8 and blood counts permitting, on day 15. After a 2 week pause the next series was started.

RESULTS

The patients have been followed for 1-17 months (mean 7) as indicated in Fig. 1. The degree of response has been evaluated according to the

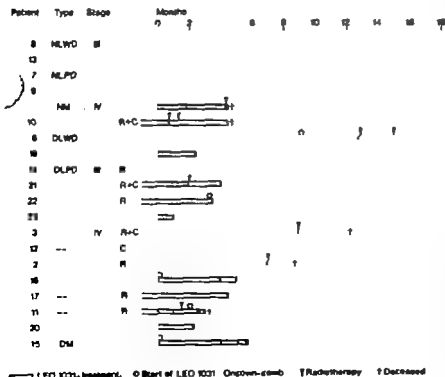


Fig. 1 Follow-up of 20 patients with lymphocytic lymphoma treated with Leo 1031. The numbering of the patient indicates the chronological order of start of treatment. R=previous radiotherapy, C=previous chemotherapy.

E.O.R.T.C. protocol (6) and the results are summarized in Table I

Complete remission was experienced by five patients (nos. 7, 8, 9, 12 and 14) and all of them are still in remission after 7–17 months (mean 1). One patient had an NLWD type of lymphoma, two had an NLPD type and two a DLDP type. Four patients were in stage III and one in the DLDP group was in stage IV.

Partial remission (>50% reduction of tumour mass for >1 month) was recorded in ten patients (nos. 1, 2, 3, 6, 13, 16, 17, 19, 20 and 23). One had an NLWD, one an NM, two a DLWD and six patients

a DLPD type of lymphoma. Two patients were in stage III and eight in stage IV. Four of the patients relapsed after 4–9 months (mean 7) whereas the other six are still in remission, 1–7 months after the start of therapy. Five of them have returned to a normal performance status. An example of the effect on splenomegaly owing to lymphomatous infiltration is presented in Fig. 2.

One patient (no. 6) displayed signs of progression after 9 months' treatment and was then started on the Leo 1031+vincristine combination schedule described. This resulted in a new remission, which was regarded as complete and is still continuing 8+ months after induction. Maintenance therapy however had to be discontinued because of a second malignancy, a well differentiated squamous cell carcinoma at the base of the tongue necessitating radiation treatment. During this period no progression of the lymphoma was observed. This group also includes one patient (no. 16) with a pleural effusion due to a well differentiated mucinous adenocarcinoma of unknown origin. Progress of this tumour was noted during treatment with the combination therapy.

Non-total failure (<50% reduction of tumour mass or partial remission for <1 month) was observed in three patients (nos. 15, 1 and 22). Two had a DLPD and one a DM type of lymphoma. Two patients were in stage III and one in stage IV. In one patient (no. 15) the disease is stationary 5+ months after induction with Leo 1031+vincristine but the other two relapsed after 2 and 3 months respectively.

Total failure was noted in two patients (nos. 10 and 11) with NM and DLPD lymphoma, respectively. In Table I the non-total failure and total failure groups are pooled in a no remission category.

During the follow-up, five patients died 3–12 months (mean 6) after starting treatment with Leo 1031 (Fig. 1). Three of them (nos. 1, 2 and 3) were treated initially in the study with a low dose.

Side-effects

Except in two cases described below, significant leucopenia has not been induced, not even after prolonged treatment. Thrombocytopenia has not been induced in any patient. Bone marrow biopsy in one patient (no. 8) displayed a normal cellular picture after more than 15 months of continuous treatment with 40 mg Leo 1031 daily.

In one patient (no. 3) a sudden drop in the WBC



Fig. 2. Scintigrams of liver and spleen (with ^{99m}Tc -sulphid colloid) in patient no. 11 with NLWD lymphoma. Cytologically confirmed involvement of the spleen. (a) At start of treatment, (b) after 3.5 months of treatment with Leo 1031 at dose level of 40 mg daily.

count was noted after 9 months therapy with a daily dose of 74 mg. This occurred however at the same time as local irradiation was administered to a small skin manifestation on the left upper arm. The WBC count was 800/ μ l as a minimum but rose within 1 week to a normal level.

In another patient (no. 12) a sudden drop in WBC to 200/ μ l was noted after 3.5 months treatment with 40 mg Leo 1031 daily. The leucopenia was observed concurrently with a transient lowering of the BP and a reversible state of mental confusion. The WBC count normalized after 2 weeks and treatment with Leo 1031 has been continued for 5+ months.

In two patients (nos. 15 and 16) for whom the cyclic schedule with Leo 1031 and vincristine was adopted as the first treatment the lowest WBC count was 1000/ μ l. The neurotoxic effects of vincristine were however already very prominent after the first two series and the combination was abandoned. Treatment was continued with Leo 1031 as a single drug therapy.

In two patients (nos. 7 and 13) receiving 40–64 mg Leo 1031 daily a cushingoid habitus developed after 8 and 4 months respectively. In one of them a tendency to oedema of the hands and development of cutaneous fibroma were also observed. Minor gastrointestinal disturbances such as a periodic diarrhoeal tendency have been observed in some of the patients.

DISCUSSION

In the present study 15 of the 20 patients with generalized LL responded to treatment with Leo 1031. The remission was complete in five patients and partial in 10. As could be expected from previous experience (1–11) the best results were obtained in patients with a nodular type of LL, whereas the response in patients with a diffuse type was less satisfactory. The average required dose was 40 mg daily.

The absence of serious side-effects permitted an ambulatory therapy in practically all the patients. This indicates that the drug is suitable in patients for whom long-term treatment with alkylating agents may be indicated. Furthermore in the event of relapse during such treatment a more aggressive regimen can be started in patients with an intact bone marrow. This relative lack of toxicity

is in accordance with published experimental observations (7).

The present preliminary results indicate a favourable effect of Leo 1031 in LL, and further trials are therefore justified. For certain types of LL a continuous therapy with Leo 1031 as a single agent may prove adequate but for others a combination therapy is probably more reliable (18). We have tried Leo 1031 as a component in a combination schedule but our experience from this mode of treatment is too limited yet to warrant any conclusions. Further attempts should therefore be made to assess the value of Leo 1031 as part of a combination chemotherapy programme.

REFERENCES

- 1 Bloomfield C, D Goldman A, Dick F, Brunning R, D & Kennedy B J. Multivariate analysis of prognostic factors in the non-Hodgkin's malignant lymphomas. *Cancer* 33: 870 1974.
- 2 Brandt L, Könyves I & Möller T. Treatment of chronic lymphocytic leukaemia and lymphosarcoma with Leo 1031: a corticosteroid-sparing with alkylating effect. Communication at 8th International Congress of Chemotherapy Athens, Abstract No B 47 1973.
- 3 Brandt L, Könyves I & Möller T. Therapeutic effect of Leo 1031: an alkylating corticosteroid ester in lymphoproliferative disorders. I. Chronic lymphocytic leukaemia. *Acta med. scand* 197: 317 1975.
- 4 Carbone P P. Non-Hodgkin lymphoma. Recent observations on natural history and intensive treatment. *Cancer* 30: 1511 1972.
- 5 Carbone P P, Kaplan H H, Musshoff B, Smithers D W & Tubiana M. Report of the Committee on Hodgkin's Disease Staging Classification. *Cancer Res* 31: 1860 1971.
- 6 E.O.R.T.C. Early Clinical Trial Cooperative Group. General protocol for co-operative chemotherapy screening study I: human cancer Dec 1973.
- 7 Eicher A, H Witz E, H R & van Patten I. M. Cell killing effectiveness of an alkylating steroid (Leo 1031). *Eur J Cancer* 9: 773 1973.
- 8 Endli J, E Z & Stutzman L. Chlorambucil therapy for lymphomas and chronic lymphocytic leukaemia. *JAMA* 191: 444 1965.
- 9 Hoogstraaten B, Owens A H, Leonard R E, Oldewell O J, Leone L A, Olson K B, Halsey J B, Townsend S R, Miller S P & Spurr C L. Combination chemotherapy in lymphosarcoma and reticulum cell sarcoma. *Blood* 33: 370 1968.
- 10 Jones S E, Fiks Z, Bull M, Kadon M E, Dorfman R F, Kaplan H S, Rosenberg S A & Kim H. Non-Hodgkin lymphoma. IV. Clinicopathologic correlation in 405 cases. *Cancer* 31: 806 1973.
- 11 Jones S E, Rosenberg S A, Kaplan H S, Kadon M E & Dorfman R P. Non-Hodgkin lymphoma. II. Single agent chemotherapy. *Cancer* 30: 31 1972.

12. Könyves, I., F. v. H. & Högborg, B. Novel corticosteroid esters with alkylating properties. Communication at 8th International Congress of Chemotherapy Athens. Abstract No. B 159 1973
13. Könyves I & Kristianson, S. Novel corticosteroid esters with alkylating properties. Communication at XIV Scandinavian Congress of Chemistry Umeå. Proc. p 187 1971
14. Leo 1031 Brochure 3rd ed. Leo Helsingborg, March 1973
15. Lowenthal, S., DeVita, V. T. & Serpick, A. A., Combined chemotherapy with nitrogen mustard, vincristine, procarbazine and prednisone in lymphosarcoma and reticulum cell sarcoma. *Cancer* 25 1018, 1970
16. Luce, J. K., Gamble, J. F., Wilson H. E., Monto R. W., Isaacs B. L., Palmer R. L., Colman, C. A., Hewlett, J. S., Gehan, E. A. & Frei, E. III. Combined cyclophosphamide, vincristine, and prednisone therapy of malignant lymphoma. *Cancer* 28 306, 1971
17. Rappaport, H., Winter W. J. & Hicks, H. B. Follicular lymphoma: reevaluation of its position in the scheme of malignant lymphomas, based on survey of 253 cases. *Cancer* 9 792, 1956.
18. Schein, E. S., Chabner B. A., Camello, J. F., Young, R. C., Bernard, C. & DeVita, V. T. Potential for prolonged disease-free survival following combination chemotherapy of non-Hodgkin lymphoma. *Blood* 43 181 1974

RENIN SECRETING RENAL TUMOUR WITH SEVERE HYPERTENSION

Case Report With Tumour Renin Analysis, Histopathological and Ultrastructural Studies

O. Ørjaviik, M. Aas, P. Fauchald, T. Hovig, B. Øystese
E. A. Brodwall and A. Flatmark

From Medical Department B, Clinical Chemical Department, Pathological Department
and Surgical Department B, Rikshospitalet
University of Oslo, Oslo, Norway

Abstract. A 25-year-old man presented with severe hypertension associated with hypokalaemia, elevated plasma renin level and secondary hyperaldosteronism. Malignant phase hypertension and renal artery stenosis were ruled out, and a preoperative diagnosis of renin-secreting renal tumour was made on the basis of higher concentrations of renin in the left than in the right renal venous plasma in spite of normal findings on selective renal arteriography. By removal of the affected kidney the tumour was found and it had a very high content of renin. Following the operation the plasma renin level, serum aldosterone concentration and BP became normal. We present a histopathological description and an ultrastructural study of the tumour.

METHODS

Plasma renin activity

PRA was measured according to Haber et al. (9) with some modifications as described by Chervu et al. (5). Blood was drawn in precooled EDTA vials. The plasma was incubated at 37°C (blank at 4°C) for 3 hours and PRA was calculated from the angiotensin I formation. The angiotensin I formed was measured by radioimmunoassay with commercial kit (Angiotensin I Immotest Kit from E. R. Squibb). Conversion of angiotensin I to angiotensin II was inhibited by the addition of EDTA, dimercaprol and hydroxyquinoline. The upper normal limit for PRA in a non-diseased person in supine position is by this method approximately 1.5 ng/ml/h (7).

Renin activity in tissue

The tissue was frozen immediately after excision. After thawing it was cut into small pieces and homogenized in Potter-Elvehjem homogenizer in 40 ml of 0.9% sodium chloride/g wet weight of tissue. After 30 min on ice with gentle movements of the pestle 6 times the homogenate was centrifuged 800×g for 5 min. The pellet was discarded and after appropriate dilution with 0.9% sodium chloride the supernatant was assayed for renin activity. Supernatant from 1.25 mg of normal kidney cortex or from $1.25 \cdot 10^{-6}$ to $1.25 \cdot 10^{-4}$ mg of the tumour was used in the assay with plasma as substrate. Blank were run with the same amounts of boiled supernatants (10 min in boiling water). Renin standard was purchased from Medical Research Centre, Holly Hill, London. One unit of renin activity gave in our hands the formation of approximately 19 000 ng angiotensin I/h.

Histological preparation

The material was fixated in buffered formal saline and Bouin fluid, processed embedded in paraffin and sectioned. The following stains were used: hematoxylin-eosin-saffron, periodic acid-Schiff reagent and Wilson stain for demonstration of the granules in the juxtaglomerular complex.

Hypertension due to renin-secreting renal tumour was first described by Robertson et al. (11) in 1967. Three months later another case was described by Kihara et al. (10) and five cases have been reported later (3, 4, 6, 8, 13). In all cases the tumour has histologically been described as hemangiopericytoma with cells resembling the juxtaglomerular cells. After surgical removal of the tumours remission of severe hypertension has occurred in all cases.

In the present report we describe a patient in whom the diagnosis was made preoperatively. Much higher renin concentrations were found in the tumour than reported in the time of investigation. Furthermore, the BP and the plasma renin activity (PRA) were closely followed during the postoperative period demonstrating a correlation between these two parameters. The tumour has also been characterized by light and electron microscopy.

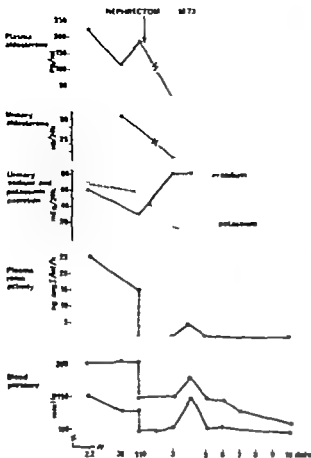


Fig. 1 Relationship between plasma aldosterone concentration, urinary aldosterone, sodium, and potassium excretion, PRA and BP before and after removal of the renin-producing tumour.

crystal studies

Specimens were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 hours and postfixed in 1% osmium tetroxide in Tyrode's solution (pH 7.4) for 4 hours.

Dehydration was carried out in graded ethanol and the material was embedded in Epon 812. Semithin and ultrathin sections were cut with an LKB ultramicrotome. The semithin sections were stained with toluidine-blue and the ultrathin sections with uranyl acetate and lead citrate. The sections were examined in a Jeol 100 B electron microscope.

CASE REPORT

The patient is a 35-year-old man previously in good health, the BP being normal in 1969. During the last two years he had suffered from episodes of headache and remarkable thirst. In Dec. 1977 he had two episodes with transient speech difficulties and paresthesia in the right hand, and an elevated BP (200/150 mmHg).

He was admitted to hospital in Jan. 1973 and apart from hypertension 180–220 mmHg systolic and 140–170 mmHg diastolic, findings of the physical examination were normal. ECG showed left ventricular hypertrophy and the chest X-ray was normal without heart enlargement. There were retinal exudates and hemorrhages with narrowing of the arterioles but not papilloedema. Serum sodium 139, potassium 4.9, chloride 96 and standard bicarbonate 37 mEq/L. Creatinine clearance was 80 ml/min and except for slight proteinuria the urine was normal. Rapid sequence I-125 pyelogram and renal arteriogram were both normal. Isotope renogram showed symmetrical, delayed excretion. Both PRA and serum aldosterone were elevated (Fig. 1).

In spite of heavy medication with hydralazine, α -methylglutamate, propranolol, trichloromethiazide and for a period spironolactone the BP was not satisfactorily regulated with diastolic values of about 110–130 mmHg while on a diet containing approximately 10 mEq sodium/day.

Bilateral renal vein catheterization was performed and very high PRA levels were found on the left side. Renin activity 1.1 units/ml/h from the right kidney was, serum 23.5 ng/ml/h and from the left kidney serum 65.3 ng/ml/h (51.9 ng/ml/h). Based on the abnormal production of renin in the left kidney the diagnosis of tumour was made and operation was advised in spite of normal findings on a new selective renal angiography with tomography.

On Oct. 1, 1973 the left kidney was removed and the tumour was found within half an hour the BP fell to normal levels (Fig. 2) and remained normal on the first postoperative days. From the 3rd to the 10th postoperative day he had a transient rise in BP (maximally 185/145 mmHg) on the 3rd postoperative day (Fig. 1). Anti-hypertensive therapy was necessary for one week and during this period the BP varied during the day and rise in BP was accompanied with doubling and tachycardia. Later the BP has been normal (110–130/73–90 mmHg) without medication for one year.

The PRA fell rapidly after removal of the left kidney (Fig. 2) with a half-life of renin in plasma of approximately 100 min. After 8 hours PRA was barely measurable. PRA followed the BP in the postoperative phase with rise up to 4.5 ng/ml/h on the 3rd postoperative day. PRA remained increased for one week and became normal simultaneously with the BP (Fig. 1). Postoperatively the serum aldosterone concentration and aldosterone excretion became normal and accordingly the urinary excretion of sodium and potassium and serum potassium concentration were normalized (Fig. 1).

RESULTS

Renin studies

The PRA was between 15 and 25 ng/ml/h on several occasions over a period of seven months (Fig. 1). At renal vein catheterization very high renin activity was found on the left side. Renin activity in the right renal vein was similar to PRA as could be expected.

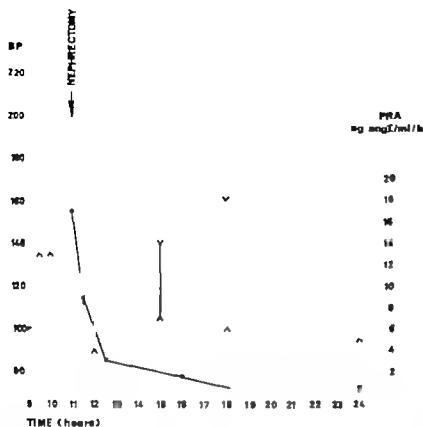


Fig 2 Relationship between PRA and BP on the day of operation



Fig 3
with

BOOK REVIEW

Trace elements in relation to cardiovascular diseases

Edited by R. Masironi. 45 pp. Sv. fr. 7.00. WHO offset Publication No. 5. Switzerland 1974

This booklet is based on the reports of three meetings (Geneva, Feb 1971, Vienna, Feb 1973 and Geneva, April 1973) of investigators on trace elements in relation to cardiovascular disease held jointly by the International Atomic Energy Agency (IAEA) and the WHO.

The subject has attracted increasing interest in recent years. WHO together with IAEA are coordinating research work on an international basis and the following six projects have been started: 1) Trace elements in human tissues in relation to ischaemic heart disease. 2) Cadmium and zinc in relation to hypertension. 3) Living population studies. 4) Trace elements in nutrition. 5) Relationships

between cardiovascular death rates and chemical characteristics of local water supplies. 6) Geochemically oriented studies in relation to cardiovascular diseases.

This booklet presents the background of these studies and the applied methodology of trace element analysis. The results to date are also presented and discussed but are still too few to be conclusive. Finally the booklet presents recommended approaches for internationally coordinated investigations on trace elements in relation to cardiovascular diseases. The booklet thus provides easily accessible information in this field of research and it is recommended not only to specialists in trace elements but also to colleagues interested in the pathophysiology of cardiovascular diseases.

P. O. Wester, Stockholm, Sweden

EDITORIALS

HIGH BLOOD PRESSURE AND PREVENTION OF STROKES

The demonstrations by the Veterans Administration Cooperative Study (6-7-8) that lowering of the blood pressure in hypertensive men caused a significant decrease in hypertension-related morbid events have led to a world-wide enthusiasm for treating patients with raised arterial pressure. This has been further stimulated by the introduction of several new BP-lowering drugs that seem to promise easier treatment with fewer side-effects. This is true even though much more experience is needed before the exact position of these drugs within the therapeutic arsenal is firmly established.

The background to the present optimism regarding treatment of high BP is well outlined in the accompanying Supplement to this issue of *Acta med. scand.* Within the general frame of prevention of some frequent disorders in this case vascular complications of arterial hypertension Ed Frelis presents a summary of the VA studies. He also demonstrates that lowering of the BP in the genetically hypertensive rat during a limited part of its lifetime is followed by a lower BP after treatment is stopped. For the rest of their lives the arterial pressure of treated rats was lower compared with their litter mates.

It is also shown that this relative decrease in BP is accompanied by less vascular changes of the arterial tree compared with those in the control group. Similar data have been published by other groups.

In this part of the symposium Ralph Reader from Australia briefly describes the present efforts all over the world to find early cases of raised BP in order to institute early treatment with the hope of preventing late vascular complications, especially cerebrovascular catastrophes. The difficulties inherent in this approach are well illustrated and the preliminary character of the experience to date is well outlined. This is also evident from the con-

tribution to the symposium from Lars Wilhelmsen who describes an ongoing Swedish study running in Göteborg. Its aim is to screen part of the middle aged male population for risk factors deemed to be of importance for the development of arterial disease and its complications. Persons identified as having risk factors of importance are offered treatment and it is planned to compare the outcome for the screened group with a control population of the same age. This study is in its 4th year and the preliminary results demonstrate in particular the prevalence of high BP in a Swedish middle aged male population, the difficulty of deciding the point at which BP should be defined as raised and the varying interest in health problems displayed by middle-aged males.

The latter problem was also emphasized by other participants in the symposium notably J. N. Morris from London who thought that a new strategy for health had to be defined, with better and more realistic health education starting early in school.

Although the results of the VA studies of treatment of high BP have been criticized the general feeling all around the world today seems to be that the time has come for a concentrated effort aiming at early treatment of high BP in large parts of the population. Whether this should be done through screening of the total community—at least above a certain age—or through other community-based programs is still an open question. The discussion provided in the accompanying Supplement to this issue of *Acta med. scand.* does not answer this or several other difficult questions. But it should serve as impetus to more official bodies responsible for health policy to consider the question.

The many studies conducted in Europe and elsewhere in conjunction with the European Office of WHO after its initiative in creating a community

the beginning of the 1950ies made it possible to produce larger quantities of antigen of different viruses. Since the major problem concerning infectious diseases in those days was poliomyelitis the interest was first focused on the possibilities to combat this disease by vaccination. Remarkable achievements have been made by the use of first the formaldehyde inactivated vaccine and later in most countries the live virus vaccine. In countries with a highly developed health care system the potential use of only inactivated vaccine to provide a medically highly safe means of immunization has been exemplified. From the global point of view poliovirus infections still present a major problem. It remains to overcome the complications posed by intercurrent enteric infections which in developing countries extensively restrict the frequency of successful immunization with live virus vaccine.

Subsequent to vaccines against poliomyelitis means for active immunization against different childhood diseases were developed. Measles was first on the list and experiences available today have clearly demonstrated that the available live virus vaccines are safe and provide a long-lasting immunity. The vaccine is extensively used in various industrialized parts of the world but there are urgent needs for the introduction of vaccine in developing countries in which measles still remains one of the most common diseases of childhood.

Rubella virus was not isolated until 1962 but within a few years attenuated vaccine strains had been developed. Vaccination against rubella has a special character since the problem concerns not primarily protect the vaccinated individual but rather the developing fetus of possible future pregnancies. Available vaccines appear harmless but there are still some questions concerning the duration of immunity and the risk for fetal damage upon accidental vaccination during pregnancy. Varying policies of vaccinations have been established in different countries. There are two major differences. The first concerns the question whether a herd immunity should be developed. The second attempts

selective protection of individuals at risk. During recent years a live attenuated mumps virus vaccine has also become available. This vaccine initiates a harmless infection and immunity as far as it has been studied appears satisfactory.

Certain negative experiences have been made in attempts to develop inactivated vaccines against different viruses of the respiratory tract. In some cases vaccination with what might have been antigenically defective products have established a worse situation than the absence of vaccination.

Further developments in this field are expected to take somewhat different courses. It should be emphasized that respiratory infections are of major importance both in developed and in developing countries.

There are many reasons to be optimistic concerning the future improvement of available and the development of new vaccines. The new vaccines will aim at giving protection against important diseases e.g. those caused by hepatitis virus, various herpes viruses and tumour viruses. Eventually we may be confronted with a relatively overloaded vaccination calendar and there will be an increasing need to arrange for simultaneous immunization against more than one disease. Still the number of future problems in providing a broad range prevention of virus diseases should not be underestimated. In each case individual evaluations will have to be made of the need for introducing a general vaccination and also of the safety of the products which may be developed. It is important that a general consensus is reached in the formulation of general principles for the application of a product.

Decisions on introducing preventive measures in the form of general vaccination carries with them considerable responsibilities. The consequences with regard to prevalence of disease and to circulation of virus in the community as well as to the chances for and the risk of challenges of the epidemiological situation need to be appreciated.

E. Norrby the Virus Department
Karolinska Institutet Stockholm Sweden

BOOK REVIEWS

Symposium on Myocardial Metabolism. Amer Heart Assoc monograph no 44 Guest Editor Eugene Braunwald. Originally published in *Circulation Research*. 215 pp \$6.00

This monograph is interesting from many points of view. It is the result of a joint meeting between American and Russian cardiologists. The president was Eugene Braunwald, Professor of Internal Medicine at Harvard. The leading Russian investigator was E. I. Chazov, Deputy Minister of Health, Moscow. The exchange of ideas was lively and the discussions are well worth printing. The subject is rather new and very sophisticated medical science that may be called molecular cardiology and the morphology is all of electron microscopic dimensions. The reviewer is reminded of a question he received from one of the professors and department heads of medicine in one of the leading American universities some 15 years ago. Who should we have as the next head of cardiology, perhaps an enzymologist?

This monograph opens up a very promising field in circulation research and demonstrates the importance of biochemical thinking also in connection with the most mechanical of all the organs, the heart pump. Disturbed homeostasis in the enzyme-dependent systems must be fundamental problem in attempting to repair the machinery.

Many of the chapters make excellent reading and it is difficult to choose some special ones. Braunwald discusses a problem of equal importance to the general practitioner and to the cardiac physiopathologist, namely the factors that may induce a reduction of the infarct size

after coronary occlusion. It is probable that clinical applications of the findings in this group may one day have decisive influence on the fate of the patient with recent infarct.

Several papers deal with the fundamental changes in protein synthesis that allow cardiac hypertrophy to take place. One group discusses the RNA changes during this process, another the metabolism of myosin both anabolic and catabolic leading to an increase in content during hypertrophy. Morgan and his group treat a similar problem with the aid of the perfused rat heart. The protein turnover has been studied in detail and the conclusion is that cardiac muscle cells appear to have a life span that may be as long as the organ itself. On the other hand molecules in this cell have a rapid turnover of 5-11 days. It is an interesting fact that the myocardial cells represent only 25% of the total cell number in the myocardium. They occupy about 75% of the total volume. The role of creatine as stimulator of amino acid incorporation into the myosin molecule is discussed. If the resistance for the left ventricle is increased it seems as if the half-life of the myosin molecules decreased to only 3 days, whereas the same value for myosin in skeletal muscle under normal conditions is 28-30 days.

Excellent electron microscopic pictures illustrate the relation of form and function on the subcellular some times even on the molecular level. It is impossible to quote more papers from this fascinating volume that allows visits that stretches far into the future of medicine in general and especially into the cardiology of new generations of investigators and practicing physicians.

Jan G. Waldenström

Brain Dysfunction in Metabolic Disorders Edited by F. Plum. 324 pp US\$25.00 Elsevier/Excerpta Medica North-Holland, Amsterdam and New York 1974

This volume is another example of the recent interest in metabolic processes in an organ that was almost terra incognita in this respect only some decades ago. Brain dysfunction in metabolic disorders is a rather modest title for a book that treats so many problems of important metabolic dysfunction of the brain. The reason, why study of disturbed metabolic homeostasis has been the basis for nearly all great improvements in medical therapy is evident. Biochemical imbalances may often be adjusted either by addition or removal of identifiable substances. One of the reasons why so many serious disturbances of brain function have been regarded as intractable has been the passive acceptance of disturbed circulation as the most

probable basis with the consecutive diagnosis cerebral sclerosis.

This book strikes a much more optimistic note discussing neurological changes in liver disease, the importance of ions, water and toxins, different metabolic disorders reflecting brain function and finally an analysis of lead intoxication.

The acceptance of hepatic coma as a metabolic intoxication has led to some remarkable improvements in therapy. These are discussed but it is also stressed that there are still many enigmas. One of them is analysed by Cavanagh who is member of the British Medical Research Council group in applied neurobiology. He studies the connection between the role of the astrocytes of glial cell and the clinical picture seen in patients with chronic severe liver disease. There are obviously characteristic morphologic changes in this cell that are connected with

liver insufficiency. It is tempting to assume that these changes are related to cerebral dysfunction. How this fits into the picture of control of ammonium ions is still enigmatic but many hepatic problems now have both a biochemical and a morphological substrate.

Another chapter treats the disturbed amino acid pattern in severe hepatic failure and tries to connect this disturbance with decreased synthesis of noradrenaline in the brain. It is possible that changes in neurotransmitters in the basis of some cerebral symptoms in hepatic encephalopathy.

Several chapters treat the problem of brain dysfunction when the circulation does not offer sufficient basic metabolites such as oxygen and glucose. Slejšö and his collaborators have an excellent chapter on ischemia and hypoxia. It is interesting to note that both these monographs treating the metabolism of heart and brain make such a clear distinction between ischemia and hypoxia. Slejšö stresses the point that the energy stores in the brain are relatively low and the tissue is therefore dependent on continuous supply of blood. It is difficult to study pure hypoxia and its effect on the brain as ischemia is usually also present. Further study of purely anoxic cell damage requires carefully controlled studies.

I will only discuss a few more of the many interesting chapters. Fishman treats the complicated problem of ion pumps when he discusses the effects of hyperosmolality and hyposmolality on the brain. This is a problem that has a very great clinical importance and we have become increasingly aware of hyponatremic coma and the ways of correcting the basic effects. It is clear that brain cell behave as modified osmometers. These problems are

further treated in the following chapter about uremia with water retention and seizures, the tetany of hypocalcemia and the stupor of hyponatremia. Two chapters are devoted to a genetic disorder causing severe neuro-encephalopathy namely the three different acute porphyrias. Recent American investigations have shown that these clinical pictures are caused by a metabolic block in the transformation of porphobilinogen into α -porphobilinogen. When external factors such as drugs or estrogens induce synthesis of the rate limiting enzyme ALA synthetase a number of metabolites increase in blood and tissues. Their role in the development of neuro-encephalopathy is still not defined but fundamental knowledge has been obtained. Also in this disease disturbances of osmolality may have severe repercussions on brain function. It seems to me that these two chapters illustrate the interaction of genes, enzymes, metabolites and drugs in a perfect way and they will teach fundamental lessons, not only to the neurologist but also to the general internist.

From the point of view of world medicine the problem of malnutrition and its influence on brain development in infants has nutritional consequences. Which treats this urgent question in a short but excellent chapter.

The book may well be said to illustrate the fecundity of dynamic basic and clinical neurology and shows the diminished importance of static morphological thinking. It may be recommended to clinicians in many fields and the editor and organizer Fred Plum at New York Hospital may well be congratulated on having organized a symposium of such excellent quality.

Ja G Wadeström

The mechanism of energy transduction in biological systems. Annals of the New York Academy of Sciences, Vol. 227. Edited by David E. Green. 680 pp. \$40.00. New York 1974.

This book, like all other New York Academy of Sciences volumes, is the outcome of a conference at the Academy. The book appeared in February 1974, the conference having taken place exactly one year before.

It is certainly fitting that Dr. D. E. Green, from the Institute for Enzyme Research in Madison, served as Editor and Conference Chairman. Many of the other authors are likewise well renowned scientists in mitochondrial biochemistry ("mitochondriacs" as they sometimes facetiously call themselves). However the scope of the book is by no means limited to the mitochondria and their plant counterparts, the chloroplasts. On the contrary the main thrust is the search for unifying principles of bioenergetics in a wide sense. The conceptual framework is provided by a 40-page introductory essay by Green. He emphasizes the fundamental role of protein molecules as macromolecular devices for energy manipulations in catalytic as well as in non-catalytic interactions. Protein molecules can serve this basic function by virtue of their spring-like tertiary structure. Thus, the science of bioenergetics becomes the

study of the energy-transducing capability of proteins, i.e. their ability to convert thermal energy into biologically useful electromechanochemical energy.

The other contributors enlarge on various aspects of the living organism's incessant struggle to harness energy from the environment and convert it into useful processes, avoiding excessive heat spillage (thermalization). The relevant changes in protein conformation are described in great detail. Naturally many of the presentations dive deep into physical chemistry, thermodynamics and quantum mechanics, thereby often transcending the absorptive powers of most medical biologists.

Considerable space is allotted to considerations of the energy transactions involved in muscular contraction and in excitable membranes. Nachmansohn summarizes his long-held view of the function of acetylcholine. This molecule is seen as a signal initiating the reaction that change ion permeability in all excitable membranes. On the other hand Nachmansohn rejects the classical view of cholinergic synaptic transmission: the acetylcholine molecules do not cross the synaptic cleft, excitation of the postsynaptic membrane being caused by overbriding ion fluxes. He asserts that Loewi's classical experiments on acetylcholine release cannot be reproduced.

In general the presentations bear no particular reference to the field of medical biochemistry. An exception is the

discussion of ionophores by Pressman and de Guzman. Ionophores are biological or artificial molecules that are able to bleed small ions, such as metal cations, and carry them through biological membranes. Since biological processes are greatly dependent on transmembrane ion gradients created by ion pumping systems it is not surprising that ionophores have interesting biological effects. This has long been known to be the case with isolated mitochondria. Pressman now describes the properties of a novel ionophore with great affinity for divalent cations, but capable of carrying other metals as well and also organic ions like noradrenaline. This compound was found to have profound effects on the performance of muscle cells. The studies to date extend to the intact dog, where a considerable increase in cardiac contractility was elicited, in conjunction with moderate increase in blood pressure and a decrease in total peripheral

resistance. Thus this ionophore is seen as possible therapeutic agent, potentially superior to present inotropic agents, i.e., catecholamines and digitalis.

A medical field notable for its absence is the disturbed energetics of hyperthyroidism. The energy loss of this disorder was long felt to be a consequence of reduced coupling in mitochondrial oxidative phosphorylation causing diversion of energy from ATP synthesis in waste heat. Rather recently it has been shown that the increased heat production is the consequence of increased ion pumping in all cells. Abnormally steep sodium and potassium gradients are being upheld and the energy involved in this useless work ultimately appears as increased heat production. Certainly pathological biochemistry is no less astounding than the normal.

Jörgen Malmquist, Malmö, Sweden

Medical Journals

Printed and distributed by Almqvist & Wiksell
publishers to the Universities of Uppsala, Stockholm and Göteborg
and to the Royal Swedish Academy of Science, etc.

Acta Chirurgica Scandinavica

Editor: L. Thörn

8 issues per volume. Free supplements. Including free subscriptions to the *Scandinavian Journal of Plastic and Reconstructive Surgery* (without suppl.), the *Scandinavian Journal of Thoracic and Cardiovascular Surgery* (without suppl.) and the *Scandinavian Journal of Urology and Nephrology* (without suppl.).
Current volume 141/1975.

Sw kr 350 per volume, incl. postage

Acta Dermato Venereologica

Editor: H. Thjesson

6 issues per volume. Free supplements.
Current volume 55/1975

Sw kr 130 per volume, incl. postage

Acta Medica Scandinavica

Editor: J. Waldenström

6 issues per volume. Free supplements.
Current volume 197 198 1975

Sw kr 225 per annum (two volumes), incl. postage

Acta Obstetrica et Gynecologica Scandinavica

Editor: Axel Ingehnman-Sundberg

5 issues per volume. Free supplements.
Current volume 54/1975

Sw kr 140 per volume, incl. postage

Acta Oto Laryngologica

Editor: C.-A. Hamberger

6 issues per volume. Free supplements.
Current volumes 79-80 1975

Sw kr 100 per volume. Two volumes per annum
Sw kr 200, incl. postage

Acta Paediatrica Scandinavica

Editor: R. Zetterström

6 issues per volume. Free supplements.
Current volume 64 1975

Sw kr 175 per volume, incl. postage

International Journal of Fertility

Editor: S. J. Behrman

4 issues per volume.
Current volume 20 1975

Sw kr 120 per volume, incl. postage

International Journal of Gynecology and Obstetrics

Editor: Harold A. Kammerlitz

6 issues per volume.
Current volume 13/1975

Sw kr 110 per volume, incl. postage

Scandinavian Audiology

Editor: Björn Blöqvist

4 issues per volume. Free supplements.
Current volume 4/1975

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Infectious Diseases

Editors: Justus Sirom and Sten Winblad

4 issues per volume. Free supplements.
Current volume 7/1975

Sw kr 110 per volume, incl. postage

Scandinavian Journal of Plastic and Reconstructive Surgery

Editor: Bengt Johanson

3 issues per volume. Free supplements.
Current volume 9/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Psychology

Editor: Lars Kiebbon

4 issues per volume.
Current volume 16/1975.

Sw kr 98 per volume, incl. postage

Scandinavian Journal of Rehabilitation Medicine

Editor: Olof Hook

4 issues per volume. Free supplements.
Current volume 7/1975

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Rheumatology

Editor: Velkko Laiho

4 issues per volume. Free supplements.
Current volume 4 1975.

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Social Medicine

Editor: Gunnar Inghe

3 issues per volume. Free supplements.
Current volume 3/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Thoracic and Cardiovascular Surgery

Editor: Viking Olav Björk

3 issues per volume. Free supplements.
Current volume 9/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Urology and Nephrology

Editor: Åke Feijofors

3 issues per volume. Free supplements.
Current volume 9 1975

Sw kr 100 per volume, incl. postage

Uppsala Journal of Medical Sciences

Editor: Gunnar Ågren

3 issues per volume. Current volume 80/1975

Sw kr 70 per volume, incl. postage

Free inspection copies on request—write to

The Almqvist & Wiksell Periodical Company
Box 62, S 101 20 Stockholm Sweden

SCINTIGRAPHY WITH ^{125}I 19-iodocholesterol IN ADRENAL DISEASE*An Evaluation*

Hans Jørgensen, Nils Norman and Johan A. Sundsfjord

*From the Medical Department B and the Hormone and Isotope Laboratory
Aker Hospital, Oslo, Norway*

Abstract Adrenal scintigraphy after I. injection of ^{125}I 19-iodocholesterol has been performed in 4 patients with primary aldosteronism, 5 with Cushing's syndrome and 1 patient with pheochromocytoma. In primary aldosteronism unilateral adrenocortical adenoma was demonstrated in 2 patients, while the method failed in 1 patient to visualize a tumour that was localized by measurements of aldosterone concentrations in the adrenal veins and by adrenal venography. In 1 patient none of the methods demonstrated a tumour. In Cushing's syndrome, adrenal scintigraphy indicated bilateral adrenocortical hyperplasia in 1 patient and visualized the tumour in 2 patients with adrenocortical adenoma. In all patients with Cushing's syndrome due to unilateral adrenocortical tumour the accumulation of radioactivity in the contralateral adrenal was suppressed. However, delayed and slight accumulation of the isotope in the suppressed gland contralateral to an adrenocortical carcinoma was misinterpreted and led to exploration on the wrong side since the tumour did not concentrate radioactivity at all. The method failed in 1 patient to localize the adrenocortical tissue responsible for the relapse of Cushing's syndrome after bilateral adrenalectomy for hyperplasia. In the patient with pheochromocytoma, no radioactivity was found on the tumour side due to compression of the adrenal cortex by the tumour. It is concluded that adrenal scintigraphy is a safe and valuable method for localization of adrenal tumours and their differentiation from adrenocortical hyperplasia. Some diagnostic pitfalls do, however, exist, as demonstrated in this series of patients.

The final diagnosis of Cushing's syndrome and of primary aldosteronism is based on hormone assays. In Cushing's syndrome it may also be possible from suppression tests to differentiate between adrenocortical tumour and hyperplasia, whereas this cannot be done in primary aldosteronism (18). It has been shown that bilateral adrenocortical hyperplasia is present in 15-30% of cases with

primary aldosteronism (6, 9, 10, 11) and that these patients rarely benefit from surgical treatment (6, 11).

The scintigraphic procedure of Conn et al. (9, 13) with visualization of the adrenal glands after ^{125}I 19-iodocholesterol administration, would in patients with Cushing's syndrome mainly be of importance for tumour localization, while in patients with primary aldosteronism the additional information with regard to the presence of a tumour or bilateral hyperplasia would be significant.

In a preliminary report (21) we presented encouraging results in two patients with primary aldosteronism in whom a unilateral adenoma was visualized by adrenal scintigraphy thus making elective surgery possible. Further experience with the method has made it clear that the results are not always so unequivocal as in our first two cases. Our total series of 10 cases may be of interest in this connection, pointing to some pitfalls that can complicate the interpretation of adrenal scintigraphy.

METHODS

Plasma cortisol was measured fluorometrically (14), plasma aldosterone (20) and plasma renin activity (19) by radioimmunochemical methods. The determination of aldosterone secretion rate was performed as described by Aakvaag (1). Urinary 17-hydroxycorticosteroids were determined by the Norymberska procedure (3) with the modifications described by Metcalf (16). Adrenal scintigraphy was performed as described previously (21). Pictures were taken with gamma camera during 4-10 days period following I. administration of ^{125}I -19-iodocholesterol. The localization of the adrenals in relation to the kidneys was established by gamma camera pictures of the latter during ^{125}I -iodohippuran renography.

Table 1 Aldosterone secretion rate (ASR) plasma aldosterone concentration (PA) and plasma renin activity (PRA) in 4 cases with primary aldosteronism

Case no.	Sex	Age (y)		ASR	PA	PRA	
				($\mu\text{g}/24 \text{ h}$) Supine	(ng/ml) Supine	(ng mg l/ml/h) Supine	Upright
1	♀	56	Preoperatively				
			Sodium depletion	345	186	0.3	0.9
			Sodium loading	503	205		
			Postoperatively		15		1.6
2	♀	51	Preoperatively				
			Sodium depletion	352	86	0.2	0.3
			Sodium loading	725	108		
			Postoperatively		34		0.8
3	♀	23	Preoperatively				
			Sodium depletion	186	208	0.1	0.1
			Sodium loading	76	133		
			Postoperatively		47		1.1
4	♂	16	Sodium depletion	271	500	0.1	0.1
			Sodium loading	254	171		
Normal values (upright, unrestricted diet)				75-150	25-150	<1.2	

RESULTS

Primary aldosteronism (4 cases Table 1)

All patients had arterial hypertension, hypokalaemia and low and suppressed plasma renin activity as well as an increased plasma aldosterone concentration and a high aldosterone secretion rate that were not or only incompletely suppressed by sodium chloride (12 g in divided doses on each of 3 days). The criteria for the diagnosis are given in Table 1. Cases 1 and 2 have been reported earlier

Case 1: 56 years of age. Adrenal vein catheterization was not performed as adrenal scintigraphy showed accumulation of radioactivity almost exclusively in the left adrenal which contained an adenoma, 22 mm in diameter.

Case 2

Female 51 years of age. A tumour in the right adrenal, 14 mm in diameter, was demonstrated by adrenal scintigraphy allowing elective surgical treatment. Previous catheterization of the right adrenal vein had not been successful.

Case 3

Female 23 years of age. This woman had developed arterial hypertension and hypokalaemia during the year before the examination. Both plasma concentration and aldosterone secretion rate were reduced by sodium loading, but not suppressed to the extent seen normally. Following the injection of ^{125}I -cholesterol a symmetrical accumulation of radioactivity appeared in both adrenal re-

gions (Fig. 1). Adrenal phlebography however disclosed an adenoma in the right adrenal, and the plasma concentration of aldosterone was 10 times higher in the right than in the left adrenal vein. The right adrenal, containing an adenoma 20 mm in diameter, was subsequently removed. Two months postoperatively the serum potassium concentration and BP were normal. The adrenal phlebography and



Fig. 1 Case 3 woman with primary aldosteronism and adenoma in the right adrenal. Photoscintigram (posterior view) showing symmetrical accumulation of radioactivity in both adrenal regions. The outline of the kidneys is drawn after ^{125}I -hippurate renography.

the adrenalectomy were performed at Telcmark Central Hospital, Skövde, under supervision of the head of the Medical Department, B. Knutson.

Case 4

Male, 17 years of age. Arterial hypertension and spontaneous hypokalaemia were first diagnosed two years before the present study. At adrenal scintigraphy radioactivity was symmetrically localized to both adrenals. Scintigraphy repeated after dexamethasone 2 mg/day in divided doses given 2 days before and 2 days after the injection of ^{125}I -cholesterol showed the same pattern of uptake (Fig. 2). No tumour was demonstrated in the left adrenal by adrenal venography; catheterization of the right adrenal vein was unsuccessful. Treatment with spironolactone has recently been started.

Cushing's syndrome (5 cases Table II)

All patients presented clinical signs of Cushing's syndrome. The results of the hormone assays are given in Table II.

Case 5

Female, 32 years of age. The hormone assays showed the pattern of bilateral adrenocortical hyperplasia. At adrenal scintigraphy the radioactivity accumulated symmetrically in both adrenals. Bilateral adrenalectomy was performed. The weights of the hyperplastic glands were 6.5 and 7.5 g respectively.

Case 6

Female, 39 years of age. The biochemical data, although inconclusive, were found to be compatible with the presence of an adrenocortical tumour. After injection of ^{125}I -cholesterol, a considerable accumulation of radioactivity in the right adrenal was found. No radioactivity was detected in the left adrenal, reflecting the suppressed function of this gland. The right adrenal was removed containing an adrenocortical adenoma (weight 25 g). The patient had to be treated with cortisone for several months after the operation.



Fig. 2 Case 4 man with primary aldosteronism. Photoscintigram (posterior view) after dexamethasone showing symmetrical accumulation of radioactivity on both adrenal regions. The results obtained after ^{125}I -biphenyl venography.

Case 7

Female, 27 years of age. The biochemical evaluation was inconclusive in the preoperative differentiation between adrenocortical tumour and hyperplasia. Adrenal scintigraphy disclosed accumulation of radioactivity in the left adrenal gland exclusively (the right one being suppressed (Fig. 3)). The left gland with an adenoma (weight 8 g) was removed. Cortisone had to be given for half year postoperatively.

Table II. Plasma cortisol, urinary 17-hydroxycorticosteroids (17-OHCS) and tetrahydro-11-deoxycortisol (THS) in 5 patients with Cushing's syndrome

Case no	Age (y)	Sex	Adrenocortical pathology	Plasma cortisol ($\mu\text{g}/100\text{ ml}$)				Urinary 17-OHCS ($\text{mg}/24\text{ h}$)				Basal urinary THS ($\text{mg}/24\text{ h}$)
				Basal at		8 a.m. after dexamethasone ^a		After dexamethasone ^a				
				8 a.m.	8 p.m.	I	II	Basal	I	II		
5	32	♀	Bilateral hyperplasia	25.0	18.4	15.7	9.8	40.7	19.2	7.1	0.11	
6	39	♀	Unilateral adenoma	28.1	23.6	20.4	9.3	24.3	-	16.2	0.13	
7	27	♀	Unilateral adenoma	29.7	27.5	27.0	7.4	25.1	28.5	31.6	0.13	
8	33	♂	Unilateral carcinoma	71.0	66.5	41.1	39.0	116.0	128.3	124.0	0.69	
9	51	♀	Hyperplasia (ectopic)	18.5	20.3	18.2	8.7	20.5	13.2	3.7	0.37	

I=2 mg/d. for 2 days, II=8 mg/d. for 2 days.

Table 1 Aldosterone secretion rate (ASR) plasma aldosterone concentration (PA) and plasma renin activity (PRA) in 4 cases with primary aldosteronism

Case no.	Sex	Age (y)		ASR	PA	PRA	
				($\mu\text{g}/24\text{ h}$) Supine	(pg/ml) Supine	(ng ang. I/ml/h) Supine	(ng ang. I/ml/h) Upright
1	♀	36	Preoperatively				
			Sodium depletion	345	186	0.3	0.9
			Sodium loading	503	205		
2	♀	51	Postoperatively		15		1.6
			Preoperatively				
			Sodium depletion	352	86	0.2	0.2
3	♀	23	Sodium loading	725	108		0.8
			Postoperatively		54		
			Preoperatively				
4	♂	16	Sodium depletion	186	208	0.1	0.1
			Sodium loading	76	133		1.1
			Postoperatively		47		
4	♂	16	Sodium depletion	271	500	0.1	0.1
			Sodium loading	254	171		
Normal values (upright, unrestricted diet)				75-150	25-150	<1.2	

RESULTS

Primary aldosteronism (4 cases Table 1)

All patients had arterial hypertension, hypokalaemia and low and suppressed plasma renin activity as well as an increased plasma aldosterone concentration and a high aldosterone secretion rate that were not or only incompletely suppressed by sodium chloride (12 g in divided doses on each of 3 days). The criteria for the diagnosis are given in Table 1. Cases 1 and 2 have been reported earlier

(Fig. 1). Adrenal phlebography however disclosed an adenoma in the right adrenal and the plasma concentration of aldosterone was 10 times higher in the right than in the left adrenal vein. The right adrenal, containing an adenoma 20 mm in diameter, was subsequently removed. Two months postoperatively the serum potassium concentration and BP were normal. The adrenal phlebography and

56 years of age. Adrenal vein catheterization was not performed as adrenal scintigraphy showed accumulation of radioactivity almost exclusively in the left adrenal which contained a adenoma, 22 mm in diameter

Case 2

Female, 51 years of age. A tumour in the right adrenal, 18 mm in diameter was demonstrated by adrenal scintigraphy allowing elective surgical treatment. Previous catheterization of the right adrenal vein had not been successful.

Case 3

Female, 21 years of age. This woman had developed arterial hypertension and hypokalaemia during the year before the examination. Both plasma concentration and aldosterone secretion rate were reduced by sodium loading, but not suppressed to the extent seen normally. Following the injection of ^{125}I -cholesterol a symmetrical accumulation of radioactivity appeared in both adrenal re-



Fig. 1 Case 3 woman with primary aldosteronism and adenoma in the right adrenal. Photoscintigram (posterior view) showing symmetrical accumulation of radioactivity in both adrenal regions. The outline of the kidneys is drawn after ^{125}I -hippuran renography



Fig 5 Case 10, woman with pheochromocytoma in the left adrenal. Photoscintigram (posterior view) showing concentration of radioactivity in the right adrenal region, but no accumulation on the tumour side.

tients with primary aldosteronism have however been somewhat difficult to reconcile with what is known of the adrenocortical function under normal and pathological conditions. Before discussing the present cases it may therefore be of interest to review some basic facts.

Firstly as evidenced by cortisol determinations in blood and urine the cortisol production in the adrenal cortical tissue is usually not suppressed by an aldosterone-producing tumour unless the tumour is also producing cortisol. *Secondly* since the normal cortisol secretion rate (15–20 mg/24 h) is 20–30 times above the highest aldosterone secretion rate we have measured in a patient with primary aldosteronism it is surprising that the increased uptake of radioactivity in an aldosterone-producing tumour will give rise to a detectable difference in radioactivity between the two adrenals. The observed concentration in the small adenomas of sufficient radioactive cholesterol to be of diagnostic importance is rather difficult to explain.

Irrespective of these considerations empirically the method of adrenal scintigraphy is obviously able at least in some patients to disclose the increased accumulation of radioactivity in such a tumour as was the case in patients 1 and 2. It is

however evident from the findings in patient 3 in whom the tumour was demonstrated by adrenal venography only that a symmetrical concentration of radioactivity in both adrenals does not rule out the presence of a unilateral tumour.

Conn et al. (9) have reported on a patient with primary aldosteronism and symmetrical accumulation of radioactive cholesterol in both adrenals, in whom a unilateral adenoma was visualized by adrenal scintigraphy repeated after suppression of the cortisol production with dexamethasone. The inability of dexamethasone to suppress the accumulation of radioactivity in the adrenals in case 4 could mean that the primary aldosteronism in this patient was due to bilateral adrenocortical hyperplasia. The duration and timing of dexamethasone administration in relation to the injection of radioactive cholesterol could however be a factor of importance. As adrenal radioactivity usually becomes visible from the third day after injection of ^{125}I -cholesterol and is maximal on the 6th or 7th day dexamethasone should probably be given 2 days before and at least 5–6 days after injection of the isotope to assure adequate suppression of the cortisol production. It is our opinion that until further experience is gained with scintigraphy after dexamethasone suppression catheterization with determination of plasma concentrations of aldosterone and cortisol from both adrenal veins combined with adrenal venography should be performed preoperatively in all patients with primary aldosteronism in whom adrenal scintigraphy is unable to localize a unilateral tumour even when performed during administration of dexamethasone. Possibly the diagnostic accuracy of adrenal scintigraphy could also be improved by adopting more objective methods for quantitation of the adrenal radioactivity.

Presumably the localization of an adrenal tumour as well as its differentiation from bilateral adrenocortical hyperplasia would be more accurate in patients with Cushing's syndrome. In bilateral hyperplasia a symmetrical concentration of radioactivity in both adrenals was to be expected. In the tumour cases the radioactivity is supposed to accumulate predominantly in the tumour the function of the contralateral adrenal being suppressed. It is difficult to see how these assumptions could be wrong when the glucocorticoid excess is due to bilateral adrenocortical hyperplasia. The adrenal scintigraphy in case 5 the only patient in this cate-

gory we have examined, is in agreement with this as are the results reported by Lieberman et al (13).

In the two patients with Cushing's syndrome caused by an adrenocortical adenoma, the heavy concentration of radioactivity to one adrenal left no doubt of the tumour side. In addition to the diagnostic value, the lack of accumulation of radioactivity in the contralateral adrenal implying a suppressed function, also predicted that postoperative substitution therapy with corticosteroids would be necessary.

So far our presuppositions hold true. In the patient with adrenocortical carcinoma, however, there was no scintigraphically detectable radioactivity in the large hormone-secreting tumour. A slight and delayed accumulation of ^{125}I -cholesterol in the contralateral atrophic gland was misinterpreted leading to exploration on the wrong side. The reason why radioactive cholesterol was not concentrated in the tumour probably was a high cholesterol turnover with increased synthesis and secretion of corticosteroid hormones and their precursors. The high cholesterol turnover was indicated not only by increased concentration of hormones in blood and urine but also by the finding that most of the cholesterol in the tumour was present as free non-esterified cholesterol. These observations—no accumulation of radioactivity in the adrenocortical tumour and suppression of the contralateral gland in two patients with adrenocortical carcinoma and one with adrenocortical adenoma—have also been made by others (2, 13) and are probably typical findings in tumours with a highocrine activity. Such a scintigraphical pattern presents a diagnostic pitfall and radiological methods will have to be adopted for correct side localization of the tumour.

That the method is also applicable for the localization of pheochromocytomas is demonstrated by case 10. The lack of radioactivity on the tumour side was due to compression of the adrenocortical cortex by the pheochromocytoma. Identical findings in two patients with pheochromocytoma have been reported by Anderson and Beierwaltes (2).

The visualization of adrenal remnants or ectopic adrenocortical tissue represents another problem not resolved by radiographical methods. Theoretically scintigraphy should be an ideal method and successful localization of adrenal remnants after total adrenalectomy for adrenocortical hyperplasia has been demonstrated (2, 13). Unfortunately

in our patient with relapse of Cushing's syndrome after bilateral adrenalectomy (no 9) we did not have the same success. The uptake of radioactive cholesterol in an ovarian corpus luteum cyst was mistaken for radioactivity in ectopic adrenocortical tissue. Since the ovarian hormones are synthesized from cholesterol a concentration of circulating cholesterol to the ovaries is not surprising. The important question which still remains to be settled is whether accumulation of ^{125}I -cholesterol in the gonads regularly takes place and if it could be teratogenous in fertile patients. The radiation doses to ovaries and testes given by Beierwaltes et al. (5) may seem unimportant, but further studies are needed as the calculations are based on observations in only one patient. The inability to detect any radioactivity in the genital regions in two men and five women in whom we later performed adrenal scintigraphy could indicate that the risk is negligible. We prefer however to administer ^{125}I -cholesterol early in the follicular phase in menstruating women and to perform the examination only in patients in whom the actual diagnosis is established clinically and by hormone assays.

In conclusion, we consider adrenal scintigraphy with ^{125}I 19-cholesterol to be a valuable method which in most cases of Cushing's syndrome will localize an adrenocortical tumour and distinguish tumour from bilateral adrenocortical hyperplasia. In primary aldosteronism the diagnostic accuracy of the method is less but may possibly be improved when performed under dexamethasone suppression and with more exact measurement of the radioactivity in each adrenal. When no side difference is found determinations of hormone concentrations in both adrenal veins combined with adrenal phlebography should be performed.

REFERENCES

- 1 Aakvaag, A. Gas-liquid-chromatography of aldosterone in biological fluids. *Chin. chin. Acta* 34 197 1971.
- 2 Anderson, B. G. & Beierwaltes, W. H. Adrenal imaging with radiolabeled cholesterol in the diagnosis of adrenal disorders. *Advanc. Intern. Med.* 19:327 1974.
- 3 Appleby, J. L., Gibson, G., Norymberk, J. K. & Stubbs, R. D. Indirect analysis of corticosteroids. I. The determination of 17-hydroxycorticosteroids. *Biochem. J.* 60:453 1955.
- 4 Bayliss, R. I. S., Edwards, O. M. & Starer, P. Complications of adrenal venography. *Brit. J. Radiol.* 43 531, 1970.

5. Beierwales, W. H., Sturman, M. F., Ryu, U. & Lee, R. D. Imaging functional modules of the adrenal gland with ¹²⁵I 19-iodocholesterol. *J. nucl. Med.* 13: 246, 1974.
6. Biglieri, E. G., Schambelin, M., Slaton, P. E. & Stockigt, J. R. The intercurrent hypertension of primary aldosteronism. *Circulat. Res., Suppl.* 1: 195, 1970.
7. Borkowski, A. J., Levin, S., Delcroix, C. & Klastersky, J. Equilibration of plasma and adrenal cholesterol in man. *J. appl. Physiol.* 28: 42, 1970.
8. Bucht, H., Bergström, J., Lindholmer, B., Wijnblad, H. J. & Hökfelt, B. Catheterization of the left adrenal vein for contrast injection and steroid analysis in a case of Cushing's syndrome. *Acta med. scand.* 176: 233, 1964.
9. Coon, J. W., Morita, R., Cohen, E. L., Beierwales, W. H., McDonald, W. J. & Herwig, K. R. Primary aldosteronism. Photoscanning of tumors after administration of ¹²⁵I 19-iodocholesterol. *Arch. Intern. Med.* 129: 417, 1972.
10. Ferriss, J. B., Brown, J. J., Fraser, R., Kay, A. W., Neville, A. M., O'Malley, I. G., Robertson, J. I. S., Symington, T. & Lever, A. F. Hypertension with aldosterone excess and low plasma renin. Preoperative distinction between patients with and without adrenocortical tumor. *Lancet* 2: 995, 1970.
11. George, J. M., Wright, L., Bell, N. H., Bartter, F. C. & Brown, R. The syndrome of primary aldosteronism. *Amer. J. Med.* 48: 340, 1970.
12. Jørgensen, H. & Stiris, G. Hypertensive crisis followed by adrenocortical insufficiency after adrenal phlebography in patient with Cushing's syndrome. *Acta med. scand.* 196: 141, 1974.
13. Lieberman, L. M., Beierwales, W. H., Coon, J. W., Ansari, A. N. & Nishiyama, H. Diagnosis of adrenal disease by visualization of human adrenal glands with ¹²⁵I-19-iodocholesterol. *New Engl. J. Med.* 285: 1387, 1971.
14. Mattingly, D. A simple fluorometric method for the estimation of free 11-hydroxycorticosteroids in human plasma. *J. clin. Path.* 15: 374, 1962.
15. Melby, J. C., Spark, R. F., Dale, S. L., Egdahl, R. H. & Kahn, P. C. Diagnosis and localization of aldosterone producing adenomas by adrenal vein catheterization. *New Engl. J. Med.* 277: 1090, 1967.
16. Metcalf, M. G. A rapid method for measuring 17-hydroxycorticosteroids in urine. *J. Endocr.* 26: 415, 1963.
17. Milachson, C. G. Epinephro-phlebography of benign tumours. *Acta radiol.* 8: 129, 1969.
18. Slaton, P. E., Jr., Schambelin, M. & Biglieri, E. G. Stimulation and suppression of aldosterone secretion in patients with an aldosterone-producing adenoma. *J. clin. Endocr.* 29: 239, 1969.
19. Sandsjöf, J. A. Radioimmunoologic determination of plasma renin activity during the menstrual cycle and during acute progesterone administration. *Acta endocr. (Kbh.)* 67: 174, 1971.
20. Sandsjöf, J. A. & Aakvaag, A. Variations in plasma aldosterone and plasma renin activity throughout the menstrual cycle, with special reference to the preovulatory period. *Acta endocr. (Kbh.)* 73: 499, 1973.
21. Sandsjöf, J. A., Norrman, N. & Jørgensen, H. Adrenal scintigraphy in primary aldosteronism. Preliminary report. *Acta med. scand.* 195: 15, 1974.

BONE MASS IN OBESE SUBJECTS

Nils Dalén, Dag Hallberg and Bertil Lönke

*From the Departments of Diagnostic Radiology and Surgery, Karolinska sjukhuset,
and the Department of Medical Engineering, Karolinska Institutet
Medical School, Stockholm, Sweden*

Abstract The inner and outer diameters of the cortex have been measured in 32 obese subjects in the middle of the second metacarpal bone and in the proximal part of radius. The results, which were compared with an age-matched control group, showed that the obese subjects had on an average an 11% larger cortical area than the controls ($p < 0.05$). The increased cortical area was caused by the greater outer diameter of the measured bones. There were no significant differences in inner diameters between the groups. The inner diameters increased with age in the same way in both obese and control persons, indicating that the former are not protected against osteoporosis in the form of endosteal resorption.

The question as to whether obese subjects, concomitantly with a hyperplasia or hypertrophy of the adipose tissue, also have a similar change in the skeleton was raised in an earlier publication (4). On the basis of observations in four obese subjects in that study with the X-ray spectrophotometric method, a much higher bone mineral mass content was found than in the controls.

In order to elucidate whether obese persons in general have a high bone mass, and if this could be caused by an increased periosteal apposition or a decreased endosteal resorption, the cortical dimensions in the second metacarpal bone and the proximal part of radius were measured.

MATERIAL AND METHODS

The obese subjects (22 women and 10 men) were all out patients, either on the waiting list for treatment at the Surgical Department, or operated upon with an intestinal shunt. Apart from their obesity they were in good general condition and had no other known metabolic disorder.

They had undergone general physical examination, including chest X-ray and routine laboratory blood tests.

The control group was age-matched and drawn at random from the Stockholm population. The age distribution of the material measured was as follows: Obese women, age 30-34 $n=8$, age 45-49 $n=8$, age 55-59 $n=6$. Obese men, age 33-39 $n=10$. Control women, age 30-34 $n=8$, age 45-49 $n=8$, age 55-59 $n=6$. Control men, age 33-39 $n=10$.

The measurements of bone dimensions were made at skeletal X-ray. The inner and outer diameters of the cortex were measured in the middle of the second metacarpal bone (1) and in the proximal part of radius (2). The cortical area was calculated according to the formula $\pi(D^2 - d^2)/4$, where D = outer diameter and d = inner diameter.

The precision of the radiological appraisal, which was determined by replicate determinations, was for the outer diameters of the metacarpal bone and radius 1.9 and 3.1% respectively and for the inner diameters 3.2 and 5.8% respectively (5). When testing the hypothesis that obese subjects do not have more bone than controls, one-sided paired t -tests were used.

RESULTS

The obese subjects were not significantly taller than the controls ($p > 0.05$): mean body height 166 cm (S.D. 6) and 166 cm (S.D. 6) respectively. Mean body weights were 136 kg (S.D. 22) and 83 kg (S.D. 9), respectively, which is a highly significant difference ($p < 0.001$).

The obese patients had a significantly larger cortical area than the controls ($p < 0.05$). The difference between the groups was caused mainly by greater outer diameter of the measured bones (Table I).

The groups displayed no significant differences in

Table I Dimensions of the second metacarpal bone and proximal part of radius for 22 obese and 22 control females and for 10 obese and 10 control males

The deviation for the obese patients is expressed as percentage of the mean values for the controls and the *t*-value is calculated for the means of the two samples. One-sided test

	Mean dimensions		Deviation (%)	<i>t</i> -value	<i>p</i> -value
	Obese	Control			
Cortical area (mm²)					
Metacarpal					
Females	46	41	+12	2.1	<0.05
Males	61	53	+15	2.4	<0.05
Radius					
Females	111	84	+8	1.9	<0.05
Males	126	111	+14	1.9	<0.05
Outer diameter (mm)					
Metacarpal					
Females	8.3	8.0	+4	1.9	<0.05
Males	9.9	9.3	+6	1.8	<0.05
Radius					
Females	13.3	12.5	+6	2.5	<0.05
Males	15.1	14.6	+3	1.0	>0.05
Inner diameter (mm)					
Metacarpal					
Females	3.2	3.2	0	0.3	>0.05
Males	4.3	4.4	-2	0.1	>0.05
Radius					
Females	7.7	7.1	+8	1.9	<0.05
Males	8.2	8.3	-1	0.3	>0.05

inner diameter (Table I) which increased similarly with age (Table II)

There were no significant correlations between cortical area and the following clinical variables: height, blood calcium concentration or alkaline phosphatase activity in serum for the obese subjects ($p > 0.05$).

DISCUSSION

The obese subjects had on an average an 11% larger cortical area than the controls ($p < 0.05$). This is in agreement with our earlier study (4) in which we found, on an average, an 8% higher bone mineral content of the shafts of radius and ulna in obese subjects.

Table II Inner and outer diameters of the second metacarpal bone and proximal part of radius for different age groups of women

The parameters are expressed as percentage of the mean for the youngest group

	Obese women			Controls		
	30-34	45-49	55-59	30-34	45-49	55-59
Outer diameter						
Metacarpal	100	100	98	100	108	107
Radius	100	100	105	100	105	98
Inner diameter						
Metacarpal	100	110	128	100	131	142
Radius	100	99	107	100	109	101

Skeletal hyperplasia is much more pronounced in trabecular than in cortical parts of the skeleton, but skeletal X-ray is not at present a suitable method for quantification of bone in trabecular parts. The cortical parts were therefore measured instead. Another advantage of the present method is that it permits precise observations of the outer and inner diameters of the cortical bones, the relationship of which is an estimate of the balance between periosteal apposition and endosteal resorption.

The high bone mass in obese subjects may be due to an increased apposition or a decreased bone resorption. In the present study the large cortical area in the obese subjects was mainly caused by a greater outer diameter of the measured bones (Table I). The fact that some of the obese patients had undergone a jejunoileal bypass operation which results in a malabsorption would only influence the results by yielding a reduced bone mass. This source of error is of no importance here because the obese subjects had a significantly larger cortical area.

As a sign of endosteal resorption the inner diameters increased with age in the same way although less markedly as in the controls (Table II). This indicates that the obese subjects are not protected against osteoporosis in the form of an elevated endosteal resorption with increasing age. This is, however, a cross-sectional study and further elucidation of this problem calls for a longitudinal study.

Little is known about the genesis of the greater cortical area in obesity. It may, however, be due to overnutrition, as was indicated in an experimental study by Hedhammar *et al.* (6) who found that overfed dogs of all ages had greater cortical thickness than controls.

Arthritis with hyperplasia is common in obese persons (7) and is usually attributed to an increased load from the body weight. The overnourished dogs, however, also had more joint complications which in the obese subjects might be caused to some extent by overnutrition in childhood. Fur-

thermore osteoporotic subjects reportedly have a tendency to be underweight, which is of great interest for this study (9).

The relationship between obesity and skeletal hyperplasia can be explained theoretically by assuming an excessive calcitonin production, as this both inhibits lipolysis in adipose tissue (10) and favours apposition of calcium in bones. Overnutrition also increases the secretion of gastrin which in turn stimulates the secretion of calcitonin (2, 3).

REFERENCES

1. Barnett, E. & Nordin, B. E. C. The radiological diagnosis of osteoporosis. A new approach. *Clin. Radiol.* 11 166, 1960.
2. Cooper C. W., Biggerstaff C. R., Wiseman, C. W. & Carlsson, M. F. Hypocalcaemic effect of pentagastrin and related gastrointestinal hormonal peptides in the rat. *Endocrinology* 91 1455 1972.
3. Cooper C. W., Schwesinger W. H., Ostjes, D. A., Mahgoub, A. M. & Manson, P. L. Stimulation of secretion of pig thyrocalcitonin by gastrin and related hormonal peptides. *Endocrinology* 91 1079 1972.
4. Dalén, N. & Hallberg, D. Bone mineral content in four obese subjects before and after intestinal stent operation. A preliminary report. *Acta chir. scand.* 140 267 1974.
5. Dalén, N. & Lämke B. Grading of osteoporosis by skeletal roentgenology and bone scanning. *Acta radiol.* 15 177, 1974.
6. Hedhammar Å., Feenag Ws, Krook, L., Schryves, H. de Labarra, Å., Wahlén J. P., Kallfelz F. A., Nissey E. A., Hietry H. F., Sheffey B. E. & Ryan, G. B. Overnutrition and skeletal disease. An experimental study in growing great Dane dogs. *The Carniel Veterinarian Symp.* 5 1974.
7. Julkunen, H., Heimonen, O. P. & Pyörälä, K. Hyperostosis of the spine in an adult population. *Ann. rheum. Dis.* 30 605 1971.
8. Meema, H. E. Cortical bone atrophy and osteoporosis as manifestation of aging. *Amer. J. Roentgenol.* 89 1287 1963.
9. Saville, P. D. Observations on 80 women with osteoporotic spine fractures. In: *Osteoporosis* (ed. U. Barry), p. 38. Grune & Stratton, New York 1970.
10. Werner S. & Low H. Inhibitory effects of calcitonin on lipolysis and ^{45}Ca accumulation in rat adipose tissue *in vivo*. *Horm. metab. Res.* 11 30 1974.

Table 1 Plasma concentrations and urinary excretion of inorganic pyrophosphate (PP_i) before and during treatment with a high phosphate intake

Days	Plasma		Urine					
	PP (μ M)	P (mM)	Vol (l)	PP (μ M)	P (mM)	PP _i (μ moles/24 h)	P (mmoles/24 h)	PP _i (ad) P (mM)
<i>Before treatment</i>								
3			2.450	82.19	7.89	201.4	19.3	10.4
1	6.26	1.22	1.675	213.30	9.94	357.3	16.7	21.5
<i>During treatment with a high phosphate intake</i>								
2	5.78	1.53	1.950	180.10	27.43	351.2	53.5	6.6
8	4.80	1.59	2.175	159.50	18.26	346.9	59.7	8.7
15	4.86	1.04	1.800	194.30	23.45	349.7	42.21	8.3
22	6.27	1.62	2.150	112.50	17.71	241.9	38.1	6.4
34	6.49	1.19						
69	8.19	1.25						
89	8.46	1.22	2.550	163.34	21.91	416.52	55.87	7.5
125	9.21	1.35	2.460	322.08	48.18	792.32	118.52	6.7

METHODS

Blood was drawn by puncture as atraumatically as possible into 5 ml heparinized plastic vessels surrounded by ice. It is important that no coagulation occurs because release of PP from platelets is well known (15). The plasma was immediately separated by centrifugation in the cold and 5 volumes of ice-cold perchloric acid were added. After standing for 15 min in the cold the precipitate was removed by centrifugation and the supernatant neutralized with ice-cold KOH. This procedure has been found to give 100% recovery of PP. The urine was treated with perchloric acid in a similar way in order to remove any inorganic pyrophosphatase activity. Such treatment of urine gave 100% recovery of PP. The analytical method for PP determination has been described elsewhere (7). The P was measured by the method of Wahler Wollenberger (16).

CASE REPORT

Previous history

The patient is a 53-year-old married woman. There is no family history of bone diseases but two brothers died in infancy from unknown causes. Her mother has old-age diabetes. One year old the patient was admitted to a sanatorium for one year because of "rickets". She did not walk until two years old. She lost her permanent teeth because of "fragile enamel" at the age of 13. She was pregnant only once but had a spontaneous abortion.

At the age of 29 and 39 cysts were removed from her ovaries. After menopause at 45 she had oestrogen/androgen (Femovirin® 1 ml) injections every month because of climacteric symptoms. Since 40 she has often suffered from headache for which she has been taking up to 4 g aspirin a day.

Actual bone and joint history

In 1960 the patient had Colles' fracture of the left wrist after adequate trauma and with prompt healing. In 1966 she developed pains in her right foot. X-ray was normal but two years later several fractures of the metatarsal bones were seen. With the exception of one year—1970—when the patient was able to resume her work in a factory she gradually developed incapacitating pains in her feet, knees, hips, low back and wrist, and successive fractures in the calcaneus, processus styloideus of the ulna and radius, both tibiae and fibulae as well as pseudofractures of both femora. A general progressive halisteresis was observed and radiological and clinical signs of arthroses in both lower extremities, as well as calcification of the spinal ligaments.

Diagnosis

Blood examinations were not carried out until 1970 when a very low alkaline phosphatase activity was found. Later in the same year a search for hyperparathyroidism was made with negative result. In 1973 hypophosphataemia was suspected because of the invariably subnormal alkaline phosphatase activities and the diagnosis was confirmed by the finding of a 24-hour urinary excretion of 244 μ moles phosphoethanolamine value far above normal (12). Traces of the same compound were found in plasma.

A bone biopsy taken in 1973 contained considerable amounts of uncalcified osteoid. Alkaline phosphatase activity was normal in biopsy from the small intestine. Histochemical examination of granulocytes for alkaline phosphatase activity gave the very low score of 4 (NR 40–100).

Other laboratory investigations

The patient's height was 155 cm, weight 55 kg, BP 140/90. Total blood count was normal. ESR 7 mm/h. Alkaline phosphatase in serum was 3–10 U/l (NR 13–38, modified

Bessey-Lowry) bioenzymic determinations were not possible, the total activity being so low. Acid phosphatase was 7.1 U/l (2.7-10.5). Alanine aminotransferase α -amylase, bilirubin and prothrombins were normal. Serum electrophoresis normal. In plasma the following concentrations were found: calcium 2.53 mM, phosphate 1.56 mM, sodium 142 mM, potassium 3.9 mM, standard bicarbonate 24.3 mM, and magnesium 0.83 mM. Serum creatinine was 0.09 mM, carbamide N 7.8 mM, creatinine clearance 0.94 ml/sec (NR 1.6-2.0). A 24-hour urine contained 4.6 mmoles calcium and 17 mmoles phosphate. There was no albumin, blood or glucose in the urine, and microscopy was normal. Traces of blood were found in her feces, 9 mmoles lipid were excreted in 24-hour feces sample.

Radiologically her chest, cranium and stomach appeared normal. Intravenous pyelograms were slightly contracted. ECGs were normal. Serum thyroxine was 95 mM. A calcium balance study showed normal results and measurements of mineral contents in bone showed low normal values.

Orthopedic treatment

At first, in 1967, when no bone or joint lesions had been found the patient's pains were interpreted as muscular and treated with physical therapy. Later, when multiple fractures were found in her right foot, this was immobilized with good result. In 1972 the patient had pains in her left knee and cras for 4 months before a fracture was diagnosed. Even 4 months of immobilization did not result in healing. In March 1973 therefore an osteosynthesis of the left tibia was performed. Increasing consolidation resulted, but one year later the fracture line was still visible. A somewhat similar course was observed for the right tibia. The fracture line was first seen in Nov. 1973 but as the patient hardly ever stood on her legs, but moved around using crutches or in wheelchair no further treatment was found necessary. However the fracture line became more and more distinct and an osteosynthesis of the right tibia was performed in May 1974.

Phosphate treatment

The patient has never received cortisone, calcium or vitamin D treatment.

A preparation of disodium hydrogen phosphate, 300 mg, and potassium dihydrogen phosphate, 200 mg, was used. The patient received 12 g of this compound, day as 8 tablets three times a day, which corresponds to an increased phosphorus intake of 1.98 g/day.

Before onset of the high phosphate intake the patient was admitted to hospital and kept on standard diet containing 1260 mg phosphorus per day in order to establish pretreatment level of plasma PP.

The plasma concentration and the urinary excretion of PP were elevated before as well as during treatment (Table 1). The normal concentration of PP in plasma is $1.92 \pm 0.092 \mu\text{M}$ (S.E.M. = 23) determined by the isotope derivative method. The excretion of PP in urine from volunteers of the same age as the patient has been found to be in the region of 30 $\mu\text{moles/24 hours}$. This is in good agreement with the findings of Fleisch and Benaz (4)

whereas the plasma values are about half the magnitude reported by Russell *et al.* (14).

The plasma P concentration increased slightly during the high phosphate intake while the urinary excretion augmented to about three times the pretreatment value (Table 1). The plasma PP concentration and the urinary excretion did not change. Consequently the PP/P ratio fell to about 7, which is still far above the ratio of approximately 0.1 found in normal subjects (4).

DISCUSSION

More than 10 cases of adult hypophosphatasia have now been described (1-9, 10-11). In most of them the disease has been misinterpreted in early childhood as rickets. There is no sex preference, though the tendency of postmenopausal women to develop osteoporosis may provoke some latent cases. Typically there are symmetric fractures and pseudofractures of the lower extremities. Most of the reported adult cases had symmetrical pseudofractures of the femoral diaphyses. Progressive halisteresis has been noted in several cases as well as calcification of the spinal ligaments and chondrocalcinosis (11). In many respects, our patient had the characteristic syndrome.

Since Fleisch and Benaz (5) showed that PP concentrations in the range 10^{-4} - 10^{-5} M effectively inhibited collagen-induced calcium phosphate precipitation *in vitro*, it is tempting to speculate that the abnormally high PP concentrations in the biological fluids in hypophosphatasia patients may prevent normal calcification. Russell (13) suggested that a high phosphate intake might lead to improvement in this disease. In a later paper (14) however he stated (without documentation) that such treatment did not change PP concentrations in plasma and urine. This is in agreement with our results and may explain why a beneficial effect in our patient has been lacking so far. It has proved impossible to increase the PP excretion by loading with P. In normal subjects an increased P excretion is accompanied by an increased PP excretion, so that the PP/P ratio remains constant (4). The renal tubular transport of PP in our patient may have been saturated and this would explain why PP circulating in an abnormally high concentration, cannot be removed from the body. In the cases reported by Bongiovanni *et al.* (2) and Guilbaud and Lathre (8) the treated children showed significant clinical and radiological improvement. Bongiovanni *et al.* found a rise in urinary pyrophosphate, which

has not been confirmed by Guilbaud and Larbre or the present work. We did not find any improvement clinically or radiologically during the treatment which may be due to our patient's age.

The isotope derivative method used in the present study for measuring PP_i is specific and very sensitive (10^{-8} M can be measured in a sample volume of 50 μ l with an S.D. less than 5%). More correct concentrations of PP_i in plasma from normal subjects (1.92 ± 0.09 μ M) and from a hypophosphatasia patient have now been established. The higher values for plasma PP_i both in normals and in hypophosphatasia reported by Russell et al. (14) must be due to a systematic error. Their conclusion that PP_i is invariably elevated in plasma and urine in this disease is confirmed by the present work. Silcox and McCarty (15) recently reported a PP_i concentration in plasma of 1.8 ± 0.06 μ M (S.E.M.) from 94 normal subjects. The essential step in their procedure is based upon a specific enzyme and is in very good agreement with our results.

REFERENCES

1. Bartter F C. Hypophosphatasia. In: The metabolic basis of inherited disease (ed. Stanbury Wyngaarden & Fredrickson), 3rd edition, p. 1293. McGraw Hill, New York, Toronto, Sydney and London 1972.
2. Bongiovanni A, M Albem, M M Root, A W Hope J W, Martino J & Spencer D M. Studies in hypophosphatasia and response to high phosphate intake. *Amer J Med. Sci.* 235: 163 1968.
3. Fersley H N & Walker P O. Studies on alkaline phosphatases: Inhibition by phosphate derivatives and the substrate specificity. *Biochem. J.* 104: 1011 1967.
4. Fleisch H & Bizaz S. De Pyrophosphatasia-

- scheidung im Harn beim gesunden Menschen. *Helv. physiol. Acta* 21: 88, 1963.
5. — Isolation from urine of pyrophosphate a calcification inhibitor. *Amer J Physiol.* 203: 671 1962.
 6. Fleisch H, Bizaz S, & Carr A. Effect of orthophosphate on urinary pyrophosphate excretion and the prevention of urolithiasis. *Lancet* 1: 1065 1964.
 7. Flodgaard H & Fløron, P.. Thermodynamic parameters for the hydrolysis of inorganic pyrophosphate at pH 7.4 as a function of Mg^{2+} , K⁺ and ionic strength determined from equilibrium studies of the reaction. *J. Biol. Chem.* 249: 3465 1974.
 8. Guilbaud, P & Larbre F. A propos d'un cas d'hypophosphatasie II forme bénigne traitée par le phosphate. *Pédiatrie* 25: 319 1970.
 9. Hosenfeld D & Hosenfeld A. Qualitative und quantitative Untersuchungen der Isoenzyme der alkalischen Serumphosphatase bei der Hypophosphatasie. *Klin. Pdiat.* 185: 437 1973.
 10. Iardou, O M., Barney D W & Fink, R L.. Hypophosphatasia in an adult. *J Bone Jt Surg* 52-A: 1477 1970.
 11. O'Duffy J D.. Hypophosphatasia associated with calcium pyrophosphate dihydrate deposits in cartilage. Report of a case. *Arthr. and Rheum.* 11: 381 1970.
 12. Rasmussen, K. Phosphorylethanolamine and hypophosphatasia. Thesis, Aarhus 1968.
 13. Russell, R. G. G. Excretion of inorganic pyrophosphate in hypophosphatasia. *Lancet* 2: 461 1963.
 14. Russell R. G. G. Bizaz, S. Donath A., Morgan, D. B & Fleisch, H.. Inorganic pyrophosphate in plasma in normal persons and in patients with hypophosphatasia, osteogenesis imperfecta, and other disorders of bone. *J. clin. Invest.* 50: 961 1971.
 15. Silcox, D. C. & McCarty H J.. Measurement of inorganic pyrophosphate in biological fluids. *J. clin. Invest.* 52: 1863 1973.
 16. Wahler H E. & Wollenberger A. Zur Bestimmung des Orthophosphats neben säure- und/oder-lablen Phosphorsäureverbindungen. *Biochem. Z.* 329: 508, 1958.

THE ACUTE EFFECT OF SODIUM CELLULOSE PHOSPHATE ON INTESTINAL ABSORPTION AND URINARY EXCRETION OF CALCIUM IN MAN

A. Berstad H Jørgensen H Frey and J H Vogt

From Medical Department B Aker H Hospital Oslo Norway

Abstract. Intestinal ^{45}Ca absorption has been determined from blood and stool radioactivity after oral administration of the isotope in nine patients before and during cellulose phosphate treatment. Oral administration of 5 g cellulose phosphate concomitant with ^{45}Ca in 100 mg "carrier" calcium as CaCl_2 decreased ^{45}Ca absorption by 80%. Cellulose phosphate, 5 g three times daily decreased urinary excretion of non-radioactive calcium by 47%. Urinary magnesium excretion decreased by 47% whereas urinary phosphorus excretion increased by 67%. Calcium and magnesium excretion in urine decreases because cellulose phosphate binds divalent cations within the GI tract. The increased phosphorus excretion is probably due to partial hydrolysis of the substance in the gut.

Hypercalcaemia is present in a significant number of patients suffering from recurrent calcium-containing renal calculi and urine calcium may play an important role in stone formation (8, 13, 15). A reduction of urinary calcium excretion has therefore been attempted in idiopathic stone formers by several therapeutic measures such as low calcium diet (15, 22) thiazide administration (24, 25), oral inorganic phosphate (3) or cellulose phosphate (5, 17, 20). Inorganic soluble phosphate was used empirically. Although it reduces urine calcium (3), the mechanism by which it affects stone formation may be more complex (15). The therapy seems rather ineffective (7) and can cause extraskeletal calcifications (6) due to absorption of phosphate. Cellulose phosphate is on the other hand essentially unabsorbable. The substance is described as an ion exchange resin with particular affinity for divalent cations (17). When given orally it is supposed to act by binding dietary calcium and thereby decreasing its intestinal absorption and urinary excretion. In metabolic balance studies cellulose

phosphate has been found to increase faecal and decrease urinary calcium excretion in some small series (5, 14, 21). More direct information on its effect on calcium absorption is scanty (17, 20).

In the present study the intestinal absorption of ^{45}Ca has been determined in patients from blood and stool radioactivity after oral administration of the isotope before and during treatment with cellulose phosphate.

MATERIAL AND METHODS

The material comprised nine patients. Six suffered from recurrent radio-opaque renal stones, one had sarcoidosis, one hyperparathyroidism and one duodenal ulcer.

Calcium absorption was measured as described by A. Ioli et al. (2) with some modifications. About 10 μCi ^{45}Ca as $^{45}\text{CaCl}_2$ in 100 mg Ca carrier as CaCl_2 was given orally in 154 mM NaCl containing 4 g polyethylene glycol (PEG MW 4000). One capsule of carnine red (0.5 g) was taken with the calcium solution. The patients were allowed free diet but fasted for 12 hours before and 2 hours after calcium administration. Two absorption tests were carried out in each individual with an interval of 2 weeks to 3 months. In one of the two tests 5 g sodium cellulose phosphate (Calcisorb® ICN) was given orally immediately after the calcium dosage. During the following two days 5 g cellulose phosphate was given 3 times daily with meals.

Blood samples were drawn 1/2, 1, 2, 3 and 4 hours after administration of the isotope. Urine was collected for 2 days and faeces until they were no longer coloured red by carnine.

Gross radioactivity of ^{45}Ca in serum and faeces was counted in the Hormone and Isotope Laboratory Aker Hospital in 2 ml samples in a gamma spectrometer with 3 inch crystal. A 2 mm lead screen was placed around the sample tube to screen out radiation from ^{45}Sc (1), the daughter isotope of ^{45}Ca which is not absorbed from the GI tract (16).

Faeces were pooled, diluted with water weighed and

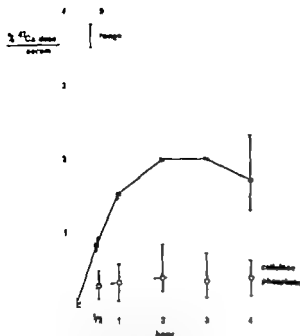


Fig 1 Serum concentration of ^{45}Ca (% of ^{45}Ca dose/l serum) after oral administration of the isotope before (●) and after (○) administration of cellulose phosphate (means and ranges).

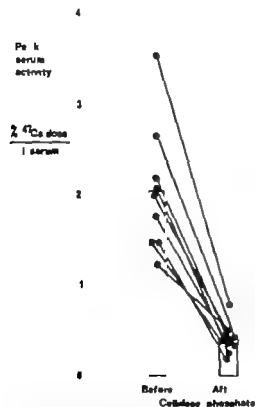


Fig 2 Peak serum concentration of ^{45}Ca (% of ^{45}Ca dose/l serum) after oral administration of the isotope. Individual values combined before and after administration of cellulose phosphate. The columns indicate means.

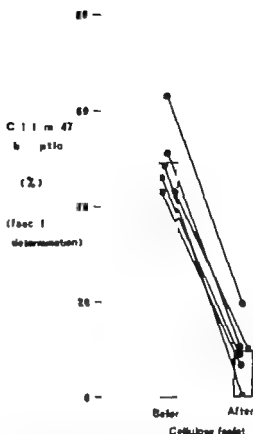


Fig 3 Net calcium absorption (%) calculated as the difference between the amount of ^{45}Ca administered and the amount of the isotope recovered in faeces corrected for loss. Individual values combined before and after administration of cellulose phosphate. The columns indicate means.

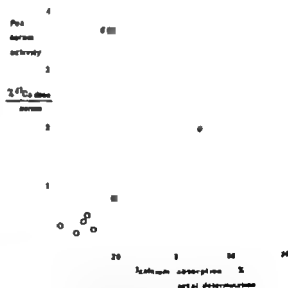


Fig 4 Relationship between ^{45}Ca absorption determined by stool and serum radioactivity. ● = values before and ○ = after administration of cellulose phosphate.

488

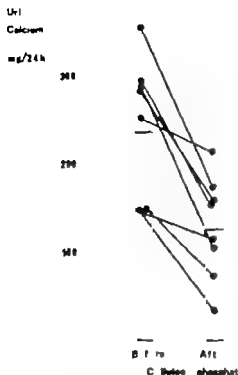


Fig. 5. Urinary excretion of non-radioactive calcium before and during cellulose phosphate treatment, 5 g three times daily. Individual values combined. Columns indicate means.

homogenized (11) before counting of ^{45}Ca in weighed samples. PEG was dissolved according to Hyden (10). PEG served as an inert unabsorbable marker to allow correction for incomplete recovery of ^{45}Ca excreted in stools, assessing similar percentage loss of unabsorbed ^{45}Ca and PEG. The assumption was tested in preliminary studies by determination of ^{45}Ca and PEG in daily stool specimens. It was found that the GI transit times of PEG and ^{45}Ca were approximately similar. The percentage loss due to incomplete collection should therefore be approximately similar for PEG and ^{45}Ca . Net absorption of ^{45}Ca was then calculated as the difference between the amounts of ^{45}Ca administered and the amounts recovered in faeces corrected for loss.

By using carmine red and collecting the red-coloured faeces, sufficient amounts of ^{45}Ca and PEG were recovered for accurate analyses without prolonging the faecal collection unnecessary. Mean recovery of PEG in the 6 patients who completed the faecal collection was 83.3% (range 56.1–112.0%). With the present method duplicate determinations of PEG on separate days in each of 23 faecal samples yielded a coefficient of variation of 3.7%.

Ca, Mg, and P in urine were determined in our Central Laboratory by routine methods, i.e. Ca and Mg by atomic absorption and P colourimetrically with molybdat

RESULTS

Without cellulose phosphate the maximal concentration of ^{45}Ca in serum was found 2, 3 and 4 hours after administration of the isotope in 4, 3 and 2 of the patients respectively. The highest serum ^{45}Ca levels were observed in the patient with sarcoidosis and the patient with hyperparathyroidism. After administration of cellulose phosphate the serum concentration of ^{45}Ca was markedly reduced in all the patients. The mean values with and without cellulose phosphate in relation to the ^{45}Ca intake are shown in Fig. 1. Peak serum concentration of ^{45}Ca , i.e. the mean of the maximal and the preceding concentration recording was reduced by cellulose phosphate on an average by 79.4% (range 59.0–88.3%) (Fig. 2).

^{45}Ca absorption determined by stool radioactivity was similarly reduced by cellulose phosphate (Fig. 3) on an average by 81.6% (range 69.0–100%).

^{45}Ca absorption determined by serum and stool radioactivity showed a good correlation ($r=0.92$) (Fig. 4).

The urinary excretion of non-radioactive calcium decreased in each subject during cellulose phosphate treatment (Fig. 5). The reduction averaged 46.8% (range 15.2–77.2%). Mean magnesium excretion in urine decreased by 47% whereas mean excretion of inorganic phosphorus increased by 67% (Table 1). The changes were found in each subject and were statistically highly significant ($p<0.001$).

Table 1. Magnesium and inorganic phosphorus in urine before (B) and during treatment (D) with sodium cellulose phosphate

Case no.	Magnesium (mEq/24 h)		Phosphorus (mg/24 h)	
	B	D	B	D
1			713	797
2	8.2	4.6	1001	1133
3	8.5	4.5	913	1343
4	4.4	1.8	480	811
5	5.7	5.0	770	1258
6	15.2	12.4	957	1467
7	8.2	6.5	1307	1929
8	5.3	2.5	705	1231
Mean	7.9	5.3	851	1249
S.D.	3.6	3.5	290	364

DISCUSSION

The estimation of intestinal calcium absorption by serum radioactivity after oral administration of ^{45}Ca suffers from the disadvantage that the serum level achieved depends on the concomitant removal of the isotope from the blood mainly into bone and urine. A more valid estimate of ^{45}Ca absorption could be obtained by determining the amount of unabsorbed ^{45}Ca excreted in faeces provided the faecal ^{45}Ca could be completely recovered. This is however practically impossible and an unabsorbable marker has to be used so that corrections can be made for incomplete collection. PEG is widely used as an inert unabsorbable marker in the GI tract, but does not appear to have been employed in clinical isotopic calcium absorption studies. It passes through the GI tract without being absorbed or split and can be determined with reasonable accuracy in biological material by a turbidimetric reaction (9, 10, 12). In the present study the marker was found to be applicable for quantitating faecal loss of ^{45}Ca , and the ^{45}Ca absorption determined by the stool radioactivity correlated well with the serum radioactivity.

The present results show that 5 g sodium cellulose phosphate effectively inhibited the intestinal absorption of ^{45}Ca when the isotope was given with 100 mg of carrier calcium as calcium chloride. The dietary calcium load is of course appreciably higher. Our normal hospital diet contains about 1100 mg calcium daily. This larger calcium load surpasses the binding capacity of cellulose phosphate making the inhibition insignificant. The reduction of non-radioactive calcium excretion in urine in the present and other studies (3, 5, 14, 21) suggests however that 5 g cellulose phosphate three times daily significantly decreases the absorption of dietary calcium as well.

Since cellulose phosphate has the property of binding divalent cations it also binds dietary magnesium (17). The urinary excretion of magnesium was consistently decreased in the present study during cellulose phosphate treatment.

Magnesium therefore has to be supplemented when cellulose phosphate is given to patients for long periods (18). The increased phosphorus excretion in urine probably means that the substance is partially hydrolyzed in the gut part of its phosphate content being absorbed (5, 18). Both magnesium and phosphate in urine may influence stone formation

(15) but to what extent cellulose phosphate affects stone formation by such mechanisms is not known.

The dependence of urinary calcium on dietary calcium (22) as well as the increased calcium absorption found in some patients with hypercalcaemia (4, 8, 19) and recurrent urinary calculi (14) suggest that the intestinal absorption of calcium may play an important role in idiopathic stone formation (15). Treatment with substances lowering calcium absorption seems therefore logical. Recently Pak et al (18) reported that cellulose phosphate was highly effective in preventing recurrent renal calculi during long-term treatment. Their findings may support the view that the initiation of stone formation is related to the precipitation of a nidus of calcium phosphate (17, 18, 19, 20) and that reduction of urinary calcium excretion by decreasing intestinal calcium absorption will reduce the propensity to stone formation. The treatment with cellulose phosphate is however somewhat inconvenient as the rather large volume of insoluble cellulose phosphate must be taken about three times daily in addition to magnesium supplement. The treatment has been found to be notably free of side-effects (18). Both hypocalcaemia, hypomagnesaemia and increased phosphate intake may lead to secondary hyperparathyroidism (23). Pak et al (18) found however that the parathyroid hormone concentration in blood was within the normal range during cellulose phosphate treatment. It is not known whether the calcium depletion will lead to a rebound increase in calcium absorption when cellulose phosphate treatment is stopped or interrupted.

REFERENCES

1. Agnew J E, Kehayoglou, A. K. & Holdsworth, C. D. Comparison of three isotopic methods for the study of calcium absorption. *Gut* 10: 590 1969.
2. Avioli, L. V., McDonald J. B., Slinger R. A. & Heanemman, F. H. A new oral isotopic test of calcium absorption. *J. clin. Invest.* 44: 128, 1965.
3. Berastein, I. S. & Newton R. The effect of oral sodium phosphate on the formation of renal calculi and idiopathic hypercalcaemia. *Lancet* 2: 1105 1966.
4. Caulfield, A., Genari, C. & Genari, L. Intestinal absorption of ^{45}Ca in stone-forming patients. *Brit. med. J.* 1: 427 1965.
5. Dent, C. E., Harper C. M. & Parfitt, A. M. The effect of cellulose phosphate on calcium metabolism in patients with hypercalcaemia. *Clin. Sci.* 27: 417 1964.

6. Dudley F J & Blackburn C. R. B. Extraskeletal calcification: complication oral neutral phosphate therapy. *Lancet* 2: 628 1970.
7. Ettinger B & Kohn F O. Inorganic phosphate treatment of nephroblastoma. *Amer J Med.* 53 32, 1973.
8. Hennessy P H., Benedict, P H., Forbes, A P & Dudley H R., Idiopathic hypercalcaemia. *New Engl J Med.* 259: 802, 1958.
9. Hyddén, S., The recovery of polyethylene glycol after passage through the digestive tract. *Ann. Agr. Coll. (Sweden)* 22, 411 1956.
10. — A turbidimetric method for the determination of higher polyethylene glycols in biological materials. *Ann. Agr. Coll. (Sweden)* 22, 139 1963.
11. Isaksson, B. Simple device for homogenizing and sampling of feces in a closed system. *Scand J clin. Lab. Invest.* 14 416, 1962.
12. Jacobson E. D. Bondy D C. Brothman, S. A. & Fordtran, J. S. Validity of polyethylene glycol in estimating intestinal water volume. *Gastroenterology* 44 761 1963.
13. Melick, R. A. & Hommesman, R. H. Clinical and laboratory studies of 207 consecutive patients in kidney-stone clinic. *New Engl. J Med.* 239: 367 1958.
14. Nassain, J. R. & Higgins B. A. Control of idiopathic hypercalcaemia. *Brit. med J* 1 675 1963.
15. Nordström, B. E. C., Metabolic bone and stone disease. Churchill Livingstone Edinburgh and London 1973.
16. Ogg, C. S. Pearson, J. D. & Vcall N. A method for measuring the gastro-intestinal absorption of ^{45}Ca using ^{45}Sc as an inert marker. *Clin. Sci.* 34 327 1968.
17. Pak, C. Y. C., Sodium cellulose phosphate. Mechanism of action and effect on mineral metabolism. *J. clin. Pharmacol.* 13 111 1973.
18. Pak, C. Y. C., Delea, C. S. & Bartter F. C. Successful treatment of recurrent nephrolithiasis (calcium stones) with cellulose phosphate. *New Engl J Med* 290 175 1974.
19. Pak, C. Y. C., East, D. A. Sazzenbacher L. J. Delea, C. S. & Bartter F. C. Gastrointestinal calcium absorption in nephrolithiasis. *J. clin. Endocr* 35 261 1972.
20. Pak, C. Y. C. Wortzman, J., Benasett, J. E. Delea, C. S. & Bartter F. C. Control of hypercalcaemia with cellulose phosphate. *J. clin. Endocr* 28 1829 1968.
21. Parfitt, A. M. Higgins, B. A., Nassim, J. R. Coddes, J. A. & Hall A., Metabolic studies in patients with hypercalcaemia. *Clin. Sci* 27 436, 1964.
22. Peacock, M., Nordström, B. E. C. & Hodgkinson, A. Importance of dietary calcium in the definition of hypercalcaemia. *Brit. med. J* 3 469 1967.
23. Reiss, E. & Canterbury J. M. Genesis of hyperparathyroidism. *Amer J Med* 50: 679 1971.
24. Yeardt, E. R., Renal calculi. *Canad. med. Ass J* 102, 479 1970.
25. Yeardt, E. R., Gossy G. F. & Garcia, D. A. The use of theobromides in the prevention of renal calculi. *Canad. med. Ass. J* 103 614 1970.

SERUM CALCITONIN RESPONSE TO INDUCED HYPERCALCEMIA

A Diagnostic Aid in Early Occult Medullary Thyroid Carcinoma

Margareta Telenius-Berg, Sven Ahnqvist and Birgitta Wikstedt

From the Departments of Internal Medicine University Hospital Lund and University Hospital Linköping Sweden

Abstract The rise in serum calcitonin (Δ -CT_{bas}) has been measured during hypercalcaemia induced by i.v. infusion of calcium gluconate. This calcium infusion test was used in a prospective screening for medullary carcinoma of the thyroid (MCT) in 4 families with Sipple syndrome as well as in 3 sporadic cases of MCT. In 16 normal controls Δ -CT_{bas} was -0.4 to $+0.5$ ng/ml (mean ± 2 S.D.). Δ -CT_{bas} was normal in 2 patients with chronic hypocalcaemia. In all 11 MCT patients Δ -CT_{bas} was markedly higher (mean-max 2.2-6.30 ng/ml), i.e. no false negatives were found. However in these cases, the diagnosis was already evident from basal serum calcitonin (S-CT), which up to now has been our most sensitive diagnostic technique for MCT. In first-degree relatives of patients with Sipple syndrome presented no signs of MCT. In 14 of these Δ -CT_{bas} was normal ("healthy relatives") but in 5 it was slightly elevated, intermediate between the control and the MCT patients. These 5 borderline cases were more sharply delineated from normal by Δ -CT_{bas} than by S-CT. Thus our calcium infusion test seems to be the most sensitive method for early diagnosis of occult MCT. We recommend the calcium infusion test for: (a) screening for MCT in all Sipple relatives with normal or only slightly elevated basal S-CT, (b) postoperative control in both sporadic and hereditary MCT, (c) investigation of supposed non-MCT tumours with calcitonin production.

Determination of basal serum calcitonin (S-CT) with a specific and sensitive radioimmunoassay is very useful in the diagnosis of medullary carcinoma of the thyroid (MCT) (1-4, 17). It is superior to conventional methods (clinical examination and thyroid scintiscan), fine needle biopsy (25) and determination of serum diamine oxidase activity (histaminase E.C. 1.4.3.6.) (27).

Both in normal human C cells and in medullary carcinoma cells, calcitonin (CT) secretion is stimulated by hypercalcaemia (17, 23).

In patients with very small tumours the basal level of S-CT may be normal or close to the upper

normal limit, e.g. in early cases and after non-radical surgery. In these patients determination of the S-CT response to induced hypercalcaemia might provide us with an even more sensitive method that would disclose occult, i.e. asymptomatic tumours. The purpose of this investigation was to evaluate this hypothesis.

MATERIAL

The patient material comprised three cases of sporadic MCT (I-3) and 30 members of four separate families (families I-IV) with Sipple syndrome. This is hereditary syndrome with MCT usually combined with bilateral pheochromocytoma (21, 27), which is transmitted as an autosomal dominant trait.

The Sipple families were screened prospectively for these hereditary tumours. Our screening program for MCT includes: 1) careful clinical examination, 2) thyroid scintiscan using ^{99m}Tc and/or ¹³¹I, 3) fine needle aspiration biopsy of all palpable thyroid nodules and/or lymph nodes of the neck, 4) determination of serum diamine oxidase activity (histaminase E.C. 1.4.3.6.) and 5) determination of S-CT using our radioimmunoassay method for human S-CT (1), both (a) basal level and (b) the response to induced hypercalcaemia, which is the subject of this report.

This screening program revealed 8 new cases of MCT (patients 1-3, II-1-3, III-1-2). All Sipple patients except one (II-2) as well as the sporadic cases (Table I) had their MCT diagnosis verified by surgery and histopathology using the criteria of Hazzard et al. (11) and Williams et al. (30). Patient II-2 refused surgery but his Sipple syndrome was regarded as indirectly verified as he was genetically proved carrier of the Sipple genome. He also had raised basal S-CT as well as bilateral pheochromocytomas, verified by adrenal angiography and high urine secretion of VMA and catecholamines.

Three Sipple patients (III-3-5), either operated upon for MCT were examined for occult metastases. They had no clinical signs of recurrence. Six patients were examined both before and after surgery for MCT (Table II).

Controls Sixteen healthy volunteers of either sex, 15-60 years without clinical or laboratory signs

Table I S-CT response to calcium infusion (Δ -CT) in 14 patients with MCT

Pat. no.	Sex	Age (y)	S-CT at 0	Δ -CT ₃₀	Δ -CT ₃₀₀
I.1	♂	57	7.3	10.9	
I.2	♀	54	5.1	1.7	4.2
I.3	♀	22	2.7	0.7	2.2
II.1	♂	58	5.2	5.6	9.5
II.2	♂	59	2.4	1.4	4.2
II.3	♂	33	4.9	4.4	14.4
III.1	♂	21	0	3.1	7.5
III.2	♀	21	2.7	1.0	7.4
			2.5	3.2	8.4
III.3	♀	48	3.4	1.8	12.9
III.4	♂	57	5.1	3.2	4.4
III.5	♂	55	13.4	0.6	10.6
S.1	♂	71	980	250	630
S.2	♀	78	6.7	4.9	12.1
S.3	♀	67	1.6	0.9	13.4
Upper reference limit (ng/ml)			1.04	0.43	0.90

Earlier operated on for MCT

ordered thyroid or calcium metabolism served as controls. Furthermore we examined two women (patients H.1 and H.2) with long-standing hypocalcaemia due to intestinal malabsorption. Patient H.1 was 47 years old had an intestinal radiation injury after radiotherapy against a carcinoma of the ovary and had been treated with vitamin D although insufficiently. Patient H.2 was 34 years old and probably had gluten-sensitive enteropathy. Both had serum calcium (S-Ca) levels around 3.6–3.9 mEq/l (Table III).

METHODS

Serum calcium. The radiochemical assay technique has been described in detail in a previous paper (1). S-CT levels expressed as ng/ml. Reference values 0.36–1.04 ng/ml. **Serum calcium.** S-Ca was determined by flame emissionometry.

Calcium infusion test. Treatment hypercalcaemia was induced by infusing 3.75 mmol=7.5 mEq=15 mg Ca⁺⁺/kg b wt. calcium gluconate in 1000 ml 10% invertose solution during 4 hours. All subjects were normocalcaemic before the infusion and had normal renal function. All except patient II.2 were fasting. The infusion induced a rise in S-Ca of $1.43 \pm 2 \times 0.56$ mEq/l (mean \pm S.D.). At the end of the infusion S-Ca was $6.25 \pm 1 \times 0.44$ mEq/l (mean \pm S.D.). MCT patients did not differ from the control in this respect.

S-CT was analysed in peripheral venous blood taken at 0', 60' and 40'. S-CT at 0' is the arithmetic mean of two separate samples taken at -5' and just before the start of the infusion. Zero time (0') is the start of the infusion, usually at about 10 a.m. In seven patients additional samples were taken at 30', 90', 120' and 180'. The expression "normal range" for Δ -CT₃₀₀=mean \pm 2 S.D. of healthy control. The S-CT the during induced hypercalcaemia at 1 min after the start, zero time, is denoted S-CT

RESULTS

Healthy controls

In the 16 healthy controls Δ -CT₃₀₀ was small, normal range -0.2–+0.5 ng/ml (mean \pm 2 S.D. =0.14 \pm 2 \times 0.18 ng/ml, $p < 0.01$ for the difference from zero) (Table III Fig. 1).

MCT patients

All 14 patients with MCT (11 hereditary and 3 sporadic cases) had elevated levels of basal S-CT and significantly higher Δ -CT₃₀₀ than the control group. The range of Δ -CT₃₀₀ was 2.2–630 ng/ml (Table I Figs 1 and 2).

In order to study the reproducibility of the results of calcium infusion test one patient (III.2) was examined twice at an interval of 2 months (Table I). The results differed very little.

In 7 MCT patients S-CT analyses were performed at closer intervals at 0', 30', 60', 90', 120' and 40'. In 4 of these patients the rise in S-CT was not uniform over time but showed an initial rise during the first hour (phase 1) then a flatter (phase 2) and finally a steeper rise again (phase 3) (Fig. 3).

In 2 of the 14 MCT patients (I.2 and III.2) determinations of basal S-CT and Δ -CT₃₀₀ were the sole indications for surgery, all other examinations being negative. The diagnosis of MCT was confirmed in both cases.

In 6 MCT patients calcium infusion was performed both preoperatively and within one month postoperatively in order to check the need for extended surgery (Table II). All but one (case II.1) showed a normal response to calcium infusion postoperatively (Fig. 4). This was interpreted as the result of radical surgery which was not contradicted by histologic evidence. These 5 patients have been

Table II Pre- and postoperative levels of basal S-CT and Δ -CT₃₀₀ (ng/ml) during calcium infusion in 6 patients operated on for MCT

Pat. no.	Preoperative		Postoperative	
	Basal S-CT	Δ -CT ₃₀₀	Basal S-CT	Δ -CT ₃₀₀
I.3	2.7	1.2	0.2	0.3
II.1	9.2	9.5	2.2	3.7
II.3	4.9	14.4	0.9	0
III.1	2.0	7.5	0.5	0.1
III.2	2.7	7.4	0.5	0
S.2	6.7	12.1	0.9	0.4

Table III. S-CT excretion (Δ -CT₂₄₀) (ng/ml) to calcium infusion (calcium gluconate 3.75 mmol=7.5 mEq=15 mg Ca⁺⁺/kg b.wt. 4 h) in (a) 16 healthy controls (b) 2 patients with chronic hypocalcemia and (c) 14 apparently healthy first-degree relatives of Sipple patients

	S-CT at 0'	Δ -CT ₂₄₀	Δ -CT ₃₆₀
(a) Mean \pm 2 S.D.	0.69 \pm 0.16	0.07 \pm 0.18	0.14 \pm 0.18
Range	0.36-1.01	-0.29-+0.43	-0.23-+0.50
(b) Pat. H.1	0.8	-0.1	-0.3
Pat. H.2	0.7	0	0.2
(c) Mean \pm 2 S.D.	0.70 \pm 0.16	0.00 \pm 0.08	0.04 \pm 0.19
Range	0.38-1.02	-0.16-+0.16	-0.34-+0.42

followed regularly (0-4 years) with clinical examinations and determinations of basal S-CT. They have all remained healthy with normal S-CT levels.

However in case 11 neither basal S-CT nor Δ -CT₃₆₀ became quite normal after surgery (Fig. 4). This was interpreted as consistent with the presence of remaining tumour tissue, although there was sufficient radicality of the surgical specimen according to histologic evaluation. Follow-up during two years has shown steadily rising levels of basal S-CT and Δ -CT₃₆₀ up to 14 and 44 ng/ml respectively. Clinical, roentgenological and scintigraphic examinations are normal. Selective venous catheterization with sampling for S-CT analysis shows very high S-CT levels in the right superior thyroid, both jugular azygos and hepatic veins. We interpret these biochemical findings as indicating the presence of multiple metastases with progressive growth.

Three hereditary cases (III 3-5) had previously been treated for MCT with bilateral neck lymph node metastases. They had all been operated on with total thyroidectomy bilateral "neck dissection" and postoperative external radiotherapy. Follow-up during 10, 8 and 6 years, respectively, had not revealed clinical signs of recurrence. Thus the raised levels of basal S-CT confirmed by definitely abnormal Δ -CT₃₆₀ in all three patients, were surprising (Table I). Also in these cases the S-CT levels were interpreted as indicating occult metastases obviously growing very slowly. The presence of metastases has not been verified by other means, the patients being unwilling to undergo further examinations.

Healthy relatives

Fourteen first-degree relatives of Sipple patients, having neither palpable thyroid abnormalities nor any clinical signs of pheochromocytoma, were

found to have normal basal levels of S-CT (mean \pm 2 S.D. = 0.70 \pm 0.16) and Δ -CT₃₆₀ within the normal range (range -0.2-+0.5 ng/ml mean \pm 2 S.D. = 0.04 \pm 0.19). These individuals were provisionally considered "healthy" as these levels coincide with those of our healthy controls (Table III Fig. 1).

Borderline cases

Another 5 first-degree relatives also lacking all clinical signs of the tumours of Sipple's syndrome had slightly elevated Δ -CT₃₆₀ levels within the range +0.9-+1.7 ng/ml. With regard to Δ -CT₃₆₀ these rela-

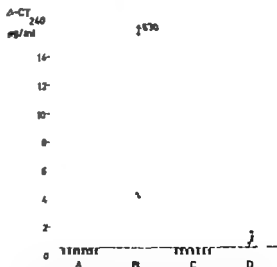


Fig. 2. S-CT response (Δ -CT₃₆₀) to induced hypercalcemia (infusion of calcium gluconate, 3.75 mmol=7.5 mEq=15 mg Ca⁺⁺/kg b.wt. 4 h). A=16 healthy controls, B=13 cases of verified MCT, C=14 apparently healthy first-degree relatives of Sipple patients, D=5 Sipple relatives classified as borderline cases.

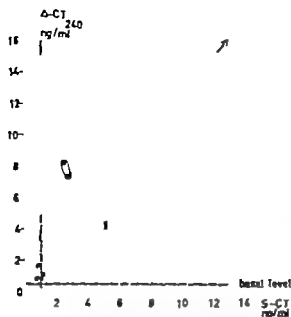


Fig 2 S-CT response to induced hypercalcemia related to basal S-CT in 13 cases of verified MCT (x) and in 5 borderline cases (●). Patient III-1 (encircled) was tested twice. Area within broken lines represents upper normal range (mean + 2 S.D.) for S-CT and Δ -CT₃₀₀.

tives were classified as borderline cases: the range of their S-CT response being intermediate between the upper border for the healthy controls and the lowest response of the verified MCT cases (0.5 and 2.2 ng/ml respectively) (Table IVa, Figs 1 and 2).

They were reexamined with another calcium infusion test about one year after the first investigation (Table IVb). At the second examination patients II 4

III 6 had developed both a higher basal S-CT and a much higher Δ -CT₃₀₀. On the basis of our series of verified MCT patients, we consider this progress to be consistent with MCT. It should be noted that the first basal S-CT in patient II 4 was also slightly elevated (1.9 ng/ml). Both patients although still without palpable tumours will be operated on with a total thyroidectomy. In the others Δ -CT₃₀₀ is still slightly elevated, most strikingly in patient III 8.

Control patients with chronic hypocalcemia

These patients were examined in order to reveal possible abnormal stores of unused CT in the C cells, which might cause an elevated S-CT response to a calcium infusion test. However, Δ -CT up to 5 hours were within normal range. Thus chronic hypocalcemia does not seem to cause false positive

results of Δ -CT as regards the diagnosis of MCT (Table III).

Determination of Δ -CT₃₀₀

As seen in Table I even Δ -CT₃₀₀ was able to discriminate all patients with verified MCT from healthy controls. But the difference from normal is still more evident if the calcium infusion is continued for 4 hours, whether Δ -CT₃₀₀ is expressed in absolute concentrations or in S.D. from normal mean.

DISCUSSION

Cancer diagnosis by a simple blood analysis has been the distant goal of much research in recent years, although as a whole with little success. A few tumours however may now be diagnosed in this way. Multiple myeloma cells produce excessive amounts of abnormal immunoglobulins, hepatic carcinoma may synthesize α -fetoprotein and carcinoma of the colon secretes carcino-embryonic antigen.

Some endocrine tumours, benign and malignant, can be revealed by blood and urine analyses showing excessive amounts of the respective hormones or degradation products thereof.

MCT is an example of such a neoplasm composed of endocrine cells with hormone synthesis. In spite of their malignant character they have retained the capacity to store and in response to hypercalcemia, release the hormone CT just like normal C cells (17-23).

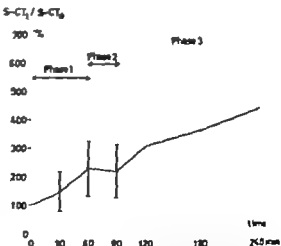


Fig 3 Relative S-CT rise during induced hypercalcemia in 7 MCT patients (mean \pm S.D.). S-CT at time 1 = S-CT_{basal} + Δ -CT.

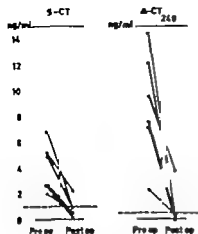


Fig. 4 Pre- and postoperative levels of basal S-CT and Δ -CT₂₄₀ in 6 patients operated on for MCT. ---upper normal reference limit.

It has been known since 1968 that MCT contains large amounts of CT and that the serum concentration of this hormone in MCT patients is very high compared with that of healthy people. Since 1969 a number of laboratories in different countries have been able to determine S-CT in MCT patients using more or less sensitive and specific radioimmunoassays (1, 2, 3, 9, 12, 23, 26) although only a few can measure normal levels (1, 12, 23, 26). This has opened new efficient ways of diagnosing MCT earlier and more safely through a simple analysis of venous blood. Thus one goal of diagnostic research has been reached for this particular tumour.

MCT is unusual in that it may occur in a hereditary form: the gene of this so-called Sipple syndrome being transmitted as an autosomal dominant trait. Thus, 50% of the children of an affected parent are expected to develop this tumour, usually in combination with bilateral pheochromocytomas. A number of these families have been reported in re-

cent years (15, 18, 21, 4). They can be considered as high risk populations for these tumours and their members should be screened regularly in order to diagnose and treat tumours as early as possible.

Unfortunately we have to wait for the neoplasm to develop before it can be diagnosed and treated. Even studies with advanced modern technique could not demonstrate chromosomal changes in Sipple's syndrome, which would help us to find the Sipple genome carrier at an earlier stage (14, 20).

The introduction of S-CT determination as a routine diagnostic screening method in these families will probably radically improve the prognosis of tumour-bearing individuals as with earlier diagnosis radical treatment can be achieved before the development of metastases.

During 1970-73 we screened three separate families with Sipple's syndrome and in 1974 we started screening a fourth Swedish family. The clinical details will be presented in a separate paper. Our family studies have proceeded parallel to those in Boston (18). Both studies show that basal S-CT is raised in most or all patients with verified MCT although very little in the earliest cases. These results are also in accordance with those published by Deftos (4).

Using the determination of basal S-CT we have been able to diagnose early multiple tumours of pinpoint to ricegrain size (patients III 1 and III 2). Basal S-CT in these cases was about 2 ng/ml (normal level ≤ 1 ng/ml). Therefore the question arises whether the determination of basal S-CT alone gives sufficient information for the clinical management of these families. Our experience is in most cases yes.

It is necessary to use a method for S-CT analysis, which is sufficiently sensitive and specific to discriminate between healthy and tumour-bearing individuals.

Table IV S-CT response (ng/ml) to calcium infusion in 5 borderline cases

Pat. no.	Age (y)	(a) First examination			(b) Second examination		
		S-CT at 0	Δ -CT ₃₀	Δ -CT ₁₈₀	S-CT at 0'	Δ -CT ₃₀	Δ -CT ₁₈₀
II:4	28	1.9	0.5	1.4	3.4	2.4	21.6
II:5	22	1.1	0	1.1	0.4	0.4	1.4
III:6	18	0.8	0.1	1.7	1.3	1.8	6.0
III:7	25	1.0	0.3	0.9	0.9	0.3	0.0
III:8	26	0.8	0.3	0.9	0.9	0.9	2.5

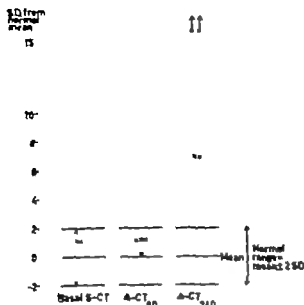


Fig 5 Basal S-CT, Δ -CT₆₀ and Δ -CT₂₄₀ in 10 calcium infusion tests in 5 borderline cases of suspected MCT

Up to now we have not found any false negative cases of *erified* MCT i.e. all patients have had raised levels of basal S-CT. The method for CT determination used by the Boston group seems to be equally sensitive and like ours (1) it allows measurements of normal levels of S-CT (26). Deftos (4) however found elevation of basal S-CT levels in only 26 of 33 patients with MCT i.e. the frequency of false negative results was as high as 21%. This illustrates the wide overlap of the diagnostic groups and pathological (i.e. MCT) caused by the sensitivity of the method.

However even when a highly sensitive and specific method is used for screening a Sipple family genetically MCT-determined individuals will be found without clinical signs of tumour but with S-CT levels just above the upper normal limit or varying around the upper reference limit. This finding, of course leaves both doctor and patient rather worried. For psychological as well as medical reasons it is desirable to be able to select the early neoplastic cases from this borderline group without having to perform a prophylactic or exploratory total thyroidectomy. This operation involves a certain risk of serious complications such as recurrent nerve lesions and hypoparathyroidism. Thus additional sensitive diagnostic methods are needed in order to separate the early neoplastic patients from their healthy relatives.

It is not known whether the individual patient, genetically determined to develop MCT, is born with an abnormal number of C cells or if there is a gradual transition from a normal number through a stage of microscopic 'C cell hyperplasia' as beautifully shown by Wolfe et al (31) to macroscopically obvious growth of multiple tumour foci.

As there seems to be a correlation between tumour size and basal S-CT as well as Δ -CT there is probably a similar evolution for S-CT and Δ -CT i.e. normal-borderline-clearly elevated levels. The concept of a range, which we now call *borderline* regarding Δ -CT₂₄₀, is a definite step forward compared to the pre-radiolimmunoassay era, when only palpable tumours could be diagnosed (see below for further discussion of the staging of MCT tumours).

As to the next goal i.e. the distinction between normal-borderline there must by definition be a statistical limit say between 2 and 3 S.D. where the high normal and the slightly pathological merge and are difficult or impossible to differentiate except as statistical probabilities. Therefore it is not certain that an increased sensitivity of the determination of S-CT could help us to make this differentiation.

Δ -CT₂₄₀ in borderline cases

Applying our new test, Δ -CT₂₄₀ (which still utilizes the same laboratory techniques as well as a subtraction with the basal S-CT) might help if there is a difference between basal S-CT and Δ -CT levels in normal and neoplastic tumour cells. Although we do not know why this difference seems to exist. Ten determinations of Δ -CT₂₄₀ in 5 patients gave markedly higher deviations from normal compared with basal S-CT both in absolute concentrations and expressed as S.D. from normal mean (Fig 5). Therefore we regard Δ -CT₂₄₀ during induced hypercalcaemia as a distinct diagnostic improvement to be applied to the most difficult patient group—the youngest patients harbouring the earliest stages of MCT.

Determination of S-CT in sporadic MCT

Also in the non-hereditary MCT the determination of basal S-CT is of great practical value as a diagnostic verification of this tumour. In our experience the primary or metastatic tumour in these cases is usually detected as a nodular goiter or an extrathyroidal neck mass. It then represents a cell mass large enough to give definitely pathological levels of basal S-CT. Thus, determination of Δ -CT₂₄₀ during calcium infusion is of no additional value.

Long-term management of MCT patients

Especially in young patients operated upon for MCT it is important to reveal and treat metastatic growth as early as possible. It is well known that this neoplasm has a propensity to metastasize early to regional lymph nodes and then to lungs and bones (6, 7, 11, 13, 16, 30). As yet no effective treatment other than radical surgery can be offered. Therefore we must intensify our diagnostic efforts not only to disclose the primary tumours at an intra-thyroidal stage, but also to detect early recurrences before spread outside regional lymph nodes has occurred. Regular postoperative determinations of $\Delta\text{-CT}_{200}$ during calcium infusion may give the same additional information as in the very early borderline cases. However it may be very difficult to localize small lymph node metastases in the fibrotic postoperative tissues without sacrificing parathyroid glands and/or recurrent nerves. Thus different supplementary methods should be tried in order to localize the metastases before explorative surgery. Such a method may be selective venous catheterization with regional sampling for S-CT analysis (10, 28) or selective arteriography of the thyroid, neck and internal mammary arteries, the last of which also supply mediastinal lymph nodes (29, 32).

Sensitivity and specificity of calcium infusion tests

Up to now we have not seen a single case of verified MCT without a significantly pathological rise of S-CT as a C cell response to induced hypercalcemia, i.e. no false negative results have been registered. *False negative* must be defined in relation to the presumed clinical stage of the patient in the evolution of Sipple MCT: 1) Non-palpable tumour and not even C cell hyperplasia. 2) Non-palpable tumour but C cell hyperplasia. 3) Non-palpable tumours of pinpoint to ricegrain size. 4) Palpable tumour (to be further subclassified according to the TMN system).

According to Melvin et al (17) and Deftos (4) MCT patients may be negative in basal S-CT but positive in $\Delta\text{-CT}$. We too find that $\Delta\text{-CT}$ is more sensitive than basal S-CT in group 2 and often in groups 3 and 4 although the suspected tumours of our borderline patients remain to be verified by histopathology.

However according to the literature the diagnostic results of calcium infusion tests have not been

entirely uniform. Three published series of MCT patients (5, 8, 26) showed varying frequencies for the occurrence of false negatives or non-responders to induced hypercalcemia, 0/13, 3/3 and 3/13 patients to be compared with our material (0/14 patients).

There are several explanations of these discrepancies e.g. (a) different sensitivity of the radioimmunoassay techniques, (b) different immunologic subspecificity of the antiserum used. Though still directed against human CT the antiserum may react with different antigenic determinants. (Note different reference values even for assays which are standardized against the same synthetic human CT M (Ciba).)

Furthermore, the evaluation of the published results is difficult as (a) The concept of $\Delta\text{-CT}$ has not been clearly defined and standardized before (b) It seems that calcium infusion tests have not been used systematically on all available MCT patients and Sipple relatives, so that at least some of the published results are in effect selected. This is also due to (c) The clinical staging of the patient material is sometimes not presented at all.

As to the response of normal C cells to hypercalcemia, it may probably be zero as in some normal controls it seems to be unmeasurable by all published S-CT assays (4, 21, 26). A low assay sensitivity per se will result in inability to measure S-CT levels in many normal subjects even after calcium infusion (4).

As yet we have seen no case of verified false positive $\Delta\text{-CT}_{200}$. False positive may be defined as to two different situations: (a) *No tumour at all*. Our control patients with long-standing hypocalcemia might have been expected to give false positive elevations of $\Delta\text{-CT}_{200}$, but both had normal responses to hypercalcemia. (b) *Non-MCT tumour with endocrine activity*. Either CT proper (ectopic production of CT with biological activity) or only cross reaction in the radioimmunoassay system.

Tumours capable of ectopic production of immunoreactive CT are notably oat cell carcinomas of the bronchus, carcinoids and other tumours of neuroectodermal origin (19, 22). In cases such as these the diagnosis of MCT should be verified or rejected. We might then possibly make use of the calcium infusion test. The determination of an increased $\Delta\text{-CT}$ during induced hypercalcemia may then be interpreted as a functional verification of a abnormal mass of biologically active C cells i.e. MCT.

It seems to be a possible hypothesis that non-MCT tumour cells with ectopic CT production would react to hypercalcaemia in an atypical way as compared with normal C cells and MCT cells. To the best of our knowledge no such examination has yet been published.

From a theoretical point of view the time course of the S-CT rise is interesting with a suggested three-phased course (Fig. 3). It is similar to that of serum insulin after a glucose load. It is reasonable to believe that the secretion processes of the two protein hormones are similar when initiated by a physiological stimulus i.e. hyperglycaemia or hypercalcaemia.

CONCLUSIONS

1 We have introduced and standardized the concept of Δ -CT₃₀₀ as the S-CT response to induced hypercalcaemia.

2 Δ -CT₃₀₀ is even more sensitive than basal S-CT for the diagnosis of early occult MCT.

3 Δ -CT₃₀₀ seems to be normal in patients with chronic hypocalcaemia.

4 We recommend the calcium infusion test for (a) screening for MCT in all Sipple relatives with normal or only slightly elevated basal S-CT. (b) postoperative control in both sporadic and hereditary MCT. (c) investigation of supposed non-MCT tumours with calcitonin production.

ACKNOWLEDGEMENT

work was supported by grants from the Swedish Cancer Society (nos. 77-40, 74-1 and 74-219).

REFERENCES

- Almqvist, S., Telenius-Berg, M. & Wåhsted, B. Serum calcitonin in medullary thyroid carcinoma. Radioimmunoassay technique and diagnostic value. *Acta med. scand.* 196, 177-194.
- Clark, M. B., Byfield, B. O. H., Boyd, O. W. & Foster, J. V. A radioimmunoassay for human calcitonin. *M. Lancet* ii, 74-1969.
- Deftos, L. J. Immunoassay for human calcitonin. I. Method. *Metabolism* 20: 1122, 1971.
- Radioimmunoassay for calcitonin in medullary thyroid carcinoma. *J. A. M. A.* 227, 403-1974.
- Deftos, L. J., Barry, A. E., Habener, J. F., Senger, F. R. & Potts, J. T. Jr. Immunoassay for human calcitonin. II. Clinical studies. *Metabolism* 20: 1129-1971.
- Dunn, E. L., Nishiyama, R. H. & Thompson, N. W. Medullary carcinoma of the thyroid gland. *Surgery* 73: 848, 1973.
- Fletcher, J. R. Medullary (solid) carcinomas of the thyroid gland. A review of 249 cases. *Arch. Surg.* 100: 257-1970.
- Frölich, M., Heersche, J. N. M., Lemkes, H. H. P. J. & Thesingh, C. W. Calcitonin secretion in medullary carcinoma of the thyroid. *Horm. metab. Res.* 4: 407-1972.
- Frölich, M., Kassenaar, A. A. H. & Smeenk, D. Radioimmunoassay of human plasma calcitonin. *Horm. metab. Res.* 3: 297-1971.
- Goltzman, D., Potts, J. T., Ridgway, E. C. & Makool, F. Calcitonin as tumor marker. *New Engl. J. Med.* 290: 1036, 1974.
- Hazard, J. H., Hawk, W. A. & Crile, G. Medullary (solid) carcinoma of the thyroid—a clinicopathologic entity. *J. clin. Endocr.* 19: 152, 1959.
- Hilfner, M. & Hesch, R. D. Radioimmunochemische Bestimmungsmethode für menschliches Calcitonin. *Klin. Wochr.* 49: 1149-1971.
- Itenez, M. L., Cole, V. W., Russell, W. O. & Clark, R. L. Solid carcinoma of the thyroid gland. *Cancer* 20: 706-1967.
- Levan, G., Mitelman, F. & Telenius, M. Chromosomes in Sipple's syndrome. *Lancet* i: 1510-1973.
- Ljungberg, O., Cederqvist, E. & Ståhlitz, W. Medullary thyroid carcinoma and pheochromocytoma: a familial chromaffinomatosis. *Brit. med. J.* i: 279-1967.
- Mandelstam, P., Rauh, B. F., Mabry, C. C. & Bartlett, R. C. "Prophylactic" thyroidectomy in a 44 year old boy with a family history of oral and ocular mucous membrane neuromas, medullary carcinoma of the thyroid, pheochromocytoma, hyperparathyroidism and diarrhea. *J. Lab. clin. Med.* 76: 867-1970.
- Melvin, K. E., W. Miller, H. H. & Tashjian, A. H., Jr. Early diagnosis of medullary carcinoma of the thyroid gland by means of calcitonin assay. *New Engl. J. Med.* 285: 1115-1971.
- Melvin, K. E., W. T. Shjian, A. H. Jr & Miller, H. H. Studies in familial (medullary) thyroid carcinoma. *Recent Prog. Hormone Res.* 28: 399-1972.
- Millhead, G., Calmette, C., Taboulet, J., Juhanov, A. & Moulkhar, M. S. Hypersecretion of calcitonin in neoplastic conditions. *Lancet* i: 452, 1974.
- Nakanishi, H., Hydozitz, J. & Sapira, J. Normal chromosomes in mucosal neuroma variant of medullary thyroid carcinoma syndrome. *J. med. Genet.* 7: 374-1970.
- Schizuka, R. M. & Hartman, W. H. Familial amyloid-producing medullary thyroid carcinoma and pheochromocytoma. A distinct genetic entity. *Ann. intern. Med.* 63: 1027-1965.
- Silva, O. L., Becker, K. L., Primack, A., Doppman, J. & Snider, R. H. Ectopic secretion of calcitonin by oat-cell carcinoma. *New Engl. J. Med.* 290: 1122, 1974.
- Silva, O. L., Snider, R. H. & Becker, K. L. Radioimmunoassay of calcitonin in human plasma. *Clin. Chem.* 20/3: 337-1974.
- Steiner, A. L., Goodman, A. B. & Powers, S. R. Study of a kindred with pheochromocytoma, medul-

- ary thyroid carcinoma, hyperparathyroidism and Cushing's disease: multiple endocrine neoplasia type 2. *Medicine* 47: 371 1968.
25. Söderström, N., Telenius-Berg, M. & Åkerman, M. Diagnosis of medullary carcinoma of the thyroid by fine needle aspiration biopsy. *Acta med. scand.* 197: 71 1973.
26. Tashjian, A. H. Jr., Howland B. G., Melvin, K. E. W. & Stratton Hill, C. Jr. Immunoassay of human calcitonin. Clinical measurement, relation to serum calcium and studies in patients with medullary carcinoma. *New Engl J Med.* 283: 890 1970.
27. Telenius-Berg, M., Ahnqvist, S. & Andersson, T. Evaluation of diagnostic methods in screening for medullary carcinoma of the thyroid. Clinical studies in families with Sipple's syndrome. To be published.
28. Telenius-Berg, M., Andersson, T. & Lunderqvist, A. Selective venous sampling for serum calcitonin. A tentative way to localize metastases of medullary thyroid carcinoma. To be published.
29. Wickham, I., Zachrisson, B. F. & Heilmann, P. Thyroid angiography. *Acta radiol.* 6: 497 1967.
30. Williams, E. III, Brown, C. L. & Doniach, L. Pathological and clinical findings in a series of 67 cases of medullary carcinoma of the thyroid. *J. clin. Path.* 19: 103 1966.
31. Wolfe, H. J., Melvin, K. E. W., Cervi-Skinner, S. J., Al-Saadi, A. A., Jølløe, J. F., Jackson, C. E. & Tashjian, A. H. Jr. C-cell hyperplasia preceding medullary thyroid carcinoma. *New Engl J Med.* 289: 437 1973.
32. Zachrisson, B. F. & Tholin, B. Personal communication.

SELECTIVE CORONARY ARTERIOGRAPHY

Björn Hoel, Harald Eke, Gudmund Semb and Egil Sivertssen

*From the Departments of Internal Medicine VIII, Radiology and Surgery III,
Ullevål Hospital, Oslo, Norway*

Abstract. A series of 414 selective coronary arteriographies in 322 patients has been done at Ullevål Hospital from May 1971 to Jan. 1974. Jodkies technique was used in all cases. The indications for arteriography, the diagnoses as regards the coronary arteries, the therapeutic consequences, and the complications of selective coronary arteriography are described.

Selective coronary arteriography was introduced at Ullevål Hospital in May 1971 with the adoption of open heart surgery. This paper presents the results of a retrospective study of 414 selective angiographies of the coronary vessels in 322 patients examined from May 1971 to the end of Jan. 1974. The review was made in order to help improve the future selection of patients for coronary arteriography in view of the diagnostic yield and the complications with which the method is associated.

METHOD

Jodkies technique for selective coronary arteriography with the transfemoral approach was used in all patients. The patient was examined after light breakfast with 0.5 mg atropine subcutaneously as premedication. The patients were not heparinized but 5 000 U heparin were added to each 1 000 ml of saline used for flushing the catheters. Left ventriculography was normally done first, followed by selective study of the left and then of the right coronary arteries. Continuous ECG and pressure recordings were done throughout the examination. The contrast media were Urografin 60% for selective arteriography and Iopaque® Corafor for ventriculography. After completion of the study the femoral artery was digitally compressed for 15 min and bag was thereafter applied and left in place for several hours and the patient was instructed to stay in bed for the rest of the day. Serial ECG and serum transaminase estimations were done whenever the patient complained of severe or long-lasting pain in connection with the procedure or when serious arrhythmias occurred.

MATERIAL

Altogether 414 examinations were done in 322 patients (Table I). The vessels examined are indicated in Table II.

In many cases selective examination of the left and right coronary arteries was done in connection with shunt angiography but this has not been included in the material. Duplicate examinations were done as indicated, for technical reasons mostly when the first examination was incomplete or inconclusive. In the Tables to follow correction for duplicate examinations has been made wherever appropriate.

The indications for coronary arteriography are shown in Table III. Coronary heart disease as total, i.e. the first six indications in Table III put together, accounted for 78% of the indications for coronary arteriography (men 84% women 61%). Valvular heart disease accounted for the remaining 22% of the indications (men 16% women 39%).

RESULTS

Diagnosis

The distribution of coronary artery disease in this study is shown in Table IV. The group "normal" comprises most of the patients with valvular disease as well as some with angina pectoris. Six of the eight patients with "shunt occluded" also had at least one shunt patent. Vice versa 6 of the 67 patients with shunt patent also had at least one shunt occluded. By "single vessel disease" is meant at least 50% stenosis of either left anterior descending, circumflex, or right coronary artery or a major branch of them. Correspondingly "double" and "triple vessel disease" refer to similar affection of two or three of these arteries. Table IV gives the relative distribution of single, double and triple vessel disease. This distribution was similar for men and women.

Of the 103 patients with normal findings or only minor changes in the coronary arteries the indication for arteriography was valvular disease in 53 cases, i.e. almost exclusively aortic valvular disease, as coronary arteriography was not often done in patients with mitral disease. In the remaining 50 cases the indication was coronary artery disease, one manifestation or another or query coronary disease.

Table I *Total material*

	No of examinations	No. of pats.	Age (y)	
			Range	Mean
Men	332	230	18-80	53
Women	III	72	25-70	57
Total	414	322		

Of the latter group many patients ultimately got the diagnosis of cardiomyopathy. Four patients with normal coronary arteries had circumstantial evidence of coronary artery disease such as previous myocardial infarction or positive ECO findings in addition to angina pectoris.

Among 55 patients studied for valvular disease alone there were 7 men and 2 women who had significant coronary artery disease (50% stenosis or more). One man was only 25 years old. He had aortic stenosis and insufficiency and a hypoplastic right coronary artery with significant atheromatous changes. He had a negative family history and no serum lipid disturbances. Another man in this group was 53 years old. The remaining 7 patients were all over 60 years of age.

A total of 97 shunts in 69 patients were studied by selective angiography. Eighty-one shunts were studied at an early stage (early shunts) i.e. before the patients left hospital after bypass surgery. Of these 75 shunts were found to be patent and 6 were occluded. One occluded and 10 patent shunts were in studied at a later stage 2-17 months post

Table II *Vessels examined*

LCA=left coronary artery RCA=right coronary artery

	Total no of examinations	Duplicate examinations
LCA+RCA	323	12
LCA	10	2
RCA	5	2
Early shunt	60	-
Late shunt	16	1
Total	414	17

operatively. The one occluded shunt remained occluded and there were no further occlusions among the patent shunts. Another 16 shunts were studied at a late stage only (late shunts) 3-17 months post-operatively. Twelve of these were found to be patent and 4 were occluded. Altogether there were 10 occluded shunts in the material. Flow was measured preoperatively in 7 of them. The flow values ranged from 20 to 50 ml/min (mean 39). Flow was measured in 84 of the 87 shunts which remained patent. It ranged from 10 to 180 ml/min (mean 77).

Therapeutic consequences

Of the 322 patients examined 125 were operated on (Table V). The remaining patients were not operated on for reasons given in Table VI.

Most of those with normal findings were patients examined for query coronary heart disease who turned out to have normal coronary arteries. Of the patients who died before operation, 3 died in relation to coronary arteriography and 6 while await-

Table III *Indications for coronary arteriography*

In some cases there was more than one indication for arteriography: figures within parentheses represent cases with only one such indication.

	No. of examinations			
	Men	Women	Total	% of total
Angina pectoris	149 (129)	34 (26)	183	41.3
CHD*	39 (38)	12 (11)	51	11.5
Unstable angina	14 (14)	2 (2)	16	3.6
Imminent infarction				
Arrhythmias	11 (8)	1 (1)	11	2.7
Cardiogenic shock	2 (2)	-	2	0.5
Aneurysm	III (17)	1 (1)	24	5.4
Valvular disease	47 (37)	3 (23)	79	17.8
Postoperative shunt angio	69 (69)	7 (7)	76	17.2

*Query coronary heart disease.

Table IV Distribution of coronary artery disease

Figures within parentheses indicate the relative distribution

Diagnosis	No. of diagnoses	Distribution (%)
Single vessel disease	54	13 (26)
Double vessel disease	80	20 (37)
Triple vessel disease	79	20 (37)
"Normal" (<30 % stenosis)	103	26
Shunt patent	67	16
Shunt occluded	8	2
Incomplete study	11	3

ing surgery. Three patients were randomized to medical treatment in a special study and therefore not operated on.

Seventeen patients underwent coronary arteriography as an emergency procedure in order to have immediate surgical treatment if possible. The indications for arteriography, the complications of arteriography and the subsequent therapeutic consequences as regards surgery as well as the ultimate fate of these patients are given in Table VII.

Complications

The serious complications of coronary arteriography in this material as registered in the case records are shown in Table VIII. The distinction between manifest and "possible myocardial infarction (AMI)" rests on the presence or absence of unequivocal pathological changes in the ECG or serum transaminases, in addition to the clinical picture of AMI. The group "serious arrhythmias" included one case of complete AV block, two cases of ventricular tachycardia, and four cases of ventricular fibrillation (VF). In addition VF occurred in 4 of the 11 patients with manifest or possible AMI.

Details of the three deaths

Case 1

A 70-year-old woman with several syncopal attacks over the past few years. She was admitted to hospital after another syncope. On admission she had atrial fibrillation which later reverted to sinus rhythm. She had aortic stenosis and mitral insufficiency and underwent left ventriculography and coronary arteriography in assessment for operative treatment. The study was completed without complications. Several hours later the patient was found to have large hematomata in the groin. By then the sand bag had been removed. She developed shock and VF and died in spite of attempted resuscitation. At autopsy there were no signs of AMI.

Table V Patients operated on

Operation	Men	Women	Total	%
Aorto-coronary bypass	11	11	22	53
Valvular repair	19	10	29	23
Aneurysmectomy	9	1	10	8
Bypass + valvular repair	4	2	6	5
Bypass + anastomosis	7	1	8	6
Total	100	25	125	100

Case 2

A 30-year-old man with mitral insufficiency and angina pectoris. On admission he had severe left ventricular failure but improved with treatment. During left ventriculography and coronary arteriography he became distressed with signs of pulmonary congestion and cerebral embolism. The study was interrupted. The patient died 3 days later. Autopsy showed a large hemorrhagic infarction in the left hemisphere.

Case 3

A 69-year-old woman with aortic and mitral valvular disease. On admission she had acute pulmonary edema. Following improvement she underwent angiography which also revealed central stenosis of the left coronary artery. The angiography had to be interrupted because the patient developed hypotension and pulmonary edema. She died 12 hours later. Autopsy showed evidence of AMI.

Of the 21 patients who developed manifest myocardial infarction (7), possible myocardial infarction (4) or serious arrhythmias (7) or died in relation to coronary arteriography (3), 20 were completely studied. Four of them had normal coronary arteries, three had single vessel disease, four double vessel disease and one triple vessel disease.

Table VI Patients not operated on

In some cases there was more than one reason for not operating; figures within parentheses represent cases with only one such reason.

Reason	Men	Women	Total	%
Technically inoperable	53 (30)	8 (8)	61	31.0
Poor risk	15 (12)	7 (6)	22	11.2
Patient refused	5	5	10	2.6
Normal findings	35	19	54	27.4
Awaiting operation	9	7	16	8.1
Symptoms too mild	25 (21)	2 (1)	27	13.7
Died before operation	4	5	9	4.5
Randomized	3	3	6	1.5

DISCUSSION

The present material showed a marked dominance of men to women among patients submitted to selective coronary arteriography. It also appeared that the men were somewhat younger than the women in this group of patients. The male dominance was even more pronounced among patients examined for coronary heart disease alone whereas there was a more even sex distribution among patients examined for valvular disease (Tables I and III). As expected angina pectoris was the single most common indication for coronary arteriography but among women it was nearly equalled by valvular disease.

Of the patients with a definite angiographic diagnosis of significant coronary artery disease only one fourth had single vessel disease. The remainder had more widespread coronary artery disease making them strong candidates for aorto-coronary bypass surgery.

The number of normal findings as regards the coronary arteries among the total number of diagnoses amounted to 25% (103 of 409). This may seem a high figure but only half of these (50 patients) were studied for coronary heart disease. In the remaining 53 cases with normal coronary arteries the indication for arteriography was valvular disease. The question arises whether or not it was justifiable to submit the patients with valvular disease to the potential hazards of selective coronary arteriography in the absence of angina pectoris. In aortic valvular disease the indication for selective coronary arteriography is twofold. Firstly there is need for exact knowledge of the anatomical relationship of the coronary arteries to the valve region; secondly there is the need to diagnose coexisting coronary artery disease. The known coexistence of aortic valvular and coronary disease due to the common underlying disease of athero-

Table VIII Complications of coronary arteriography

	Men	Women	Total	% of total material
Deaths	1	2	3	0.7
Manifest AMI	5	2	7	1.7
Possible AMI	4	—	4	1.0
Cerebrovascular accident	2	—	2	0.5
Local haematomas or bleeding	2	—	2	0.5
Serious arrhythmias	6	1	7	1.7
Femoral artery aneurysm	1	—	1	0.2

sclerosis was verified in the present material by the fact that of the 53 patients who underwent arteriography for valvular disease alone i.e. without having angina pectoris 9 patients (16%) were found to have significant coronary artery disease. Most of these patients were men and most of them were elderly.

In this material of 322 patients 4 had normal coronary angiograms in the presence of what was regarded as definite coronary heart disease. A small number of such patients must be accepted in this kind of material. Whether it may be explained by occlusion of an arterial branch flush with the main vessel by postulated small vessel disease or by faulty technique of study remains uncertain.

The relationship between shunt occlusion and low shunt flow as measured intraoperatively is well known (5). This relationship was verified in the present material by the observation that the shunts that occluded had on an average lower flow values (mean 39 ml/min) than the shunts that remained patent (mean 77 ml/min). But there was considerable variation and some of the shunts that remained patent had intraoperative flow values as low as 10–20 ml/min even after stimulation with temporary occlusion or intravascular papaverine. It is perhaps worth noticing that one of the two Y-grafts in the series occluded. In the few cases in whom repeat shunt angiographies were done no further occlusions were found on the second examination indicating that graft occlusion occurs early (2, 6).

Most of the 123 patients who came to operation had aorto-coronary vein bypass or valvular repair (Table V). The remaining 197 patients were not operated on for reasons given in Table VI. The

Table VII Patients studied as emergency cases

Indication	No of pats	Complications		Operated on	Alive
		AMI	VF		
Imminent infarction	18	2	—	7	9
Arrhythmias	5	—	1	4	4
Cardiogenic shock	2	—	—	1	2
Total	17	2	1	12	15

commonest reason for not operating was technical inoperability which accounted for 61 cases (31%) or nearly 20% of the total patient material. This figure may vary from centre to centre according to the local criteria for inoperability.

Two of the reasons for not operating require special attention. One is the situation where the patient refuses operation. Ideally no patient should undergo potentially dangerous investigations if he has already decided not to submit himself to the treatment in which the investigation is aimed. The second reason is the situation where the patient's symptoms are too mild to warrant the risk of surgery. In 22 cases this was the only reason for not operating in the present series. Clearly these patients should not have been submitted to coronary arteriography in the first place.

In a retrospective study like the present the incidence of complications of selective coronary arteriography may well be underestimated. However there is no doubt that the figures in Table VIII truly represent the actual incidence of complications as regards deaths, manifest and possible infarction, cerebrovascular accidents, whereas the true figures for haematomas and arrhythmias are probably higher than those given in the Table.

Takaro et al. (8) found that more than half of the 66 deaths occurring in 3044 coronary artery investigations were caused by acute coronary occlusion, mainly by catheter emboli. In one of our patients died from AMI there was no evidence of occlusion and the most likely mechanism was thought to be coronary artery occlusion by wedging catheter tip in the coronary ostium. In another of our patients however who died from a massive infarction a stripped-off catheter embolus have been the cause of death. In the patient who died from a local groin haematoma, death must partly be blamed on insufficient patient monitoring and this was therefore the most preventable death in the series.

Considering that all three patients who died in our series were critically ill a mortality rate of 0.7% may be acceptable in view of the important information yielded by the study.

Several authors have pointed out recently that there seems to be a marked difference in the frequency of serious complications of selective coronary arteriography between the femoral (Judkins) and the brachial (Sones) techniques. In papers comparing the two techniques mortality rates with

the femoral approach have been reported to be 2.4% (7), 1.4% (9) and 2.2% (6) in each case considerably higher than the mortality rate with the brachial method. Adams et al. (1) for instance found that the mortality related to the femoral technique was 6 times that of the brachial technique in a large nationwide survey from 173 hospitals in the US. Other serious complications were also much more common with the femoral technique. The greater tendency for complications to occur with the femoral technique has been explained partly by the increased risk of complete coronary artery occlusion by the preshaped Judkins catheters (7, 9) and partly by the need to change catheters and by the use of guide wires which in themselves have definite thrombogenic properties (4, 8). Petch et al. (7) also thought that there was a greater tendency for the Judkins catheters to stimulate the aortic baroreceptors and thereby cause reflex bradycardia and hypotension which could be fatal. On the other hand it has been claimed that the femoral technique tends to be adopted by the less skilled investigators since the coronary ostia are more readily entered by the preshaped Judkins catheters. The greater complication rate with the femoral technique seen in some centres may therefore to a large extent depend on lack of experience and technical skill (3). Not only will faulty technique in itself provoke a high incidence of complications, but undue prolongation of the study may further increase the risk of thromboembolic complications. With increasing experience the rate of complications with the femoral technique is drastically reduced. Thus Adams et al. (1) found that the mortality was reduced by a factor of 8 in centres which did more than 800 studies per 2 years as compared to centres which did less than 200 studies per 2 years. It is our conclusion, therefore, that centres which are already familiar with the femoral technique and have gained some experience in dealing with the potential hazards of coronary arteriography as we feel is the case in our hospital need not abandon the femoral technique in order to reduce the rate of complications. A change to the brachial technique may well increase rather than decrease the number of complications at least temporarily and the number of unsuccessful studies would certainly increase.

It has been pointed out that the bolus of heparin given routinely to the patients with the brachial technique may serve to reduce the number of thromboembolic complications. Judkins

(3) therefore now advocate the use of a single dose of heparin also with the femoral technique. So far this has not been the routine in our hospital.

Three of the seven patients with manifest myocardial infarction and one of the four with possible myocardial infarction associated with the arteriography in this series were successfully operated on with aorto-coronary bypass at a later stage. Therefore patients are not necessarily worse off in the end even after having sustained an infarction during selective coronary arteriography than they would have been if left uninvestigated.

One would expect a high complication rate in patients studied as emergency cases in assessment for immediate surgery. In fact such emergency arteriographies accounted for only two of the cases of definite myocardial infarction. The rest of the complications including the deaths occurred in patients studied electively. As shown in Table VII 12 of the 17 patients studied as emergency cases were operated on and 15 of the patients have survived to the present date. In properly selected patients it may therefore be well worthwhile carrying out emergency coronary arteriography and the rate of complications does not seem to be unduly high.

Takaro et al. (8) pointed out that the great majority of patients who died in connection with selective coronary arteriography had triple vessel disease. The present study confirms the high frequency of widespread disease among patients with serious implications but it also appeared that some of these patients had single vessel disease only or even normal coronary arteries.

Selective coronary arteriography is associated with a definite incidence of serious complications as shown by this and other studies. It is therefore evident that candidates for coronary arteriography must be carefully selected. The patients who will refuse operation and those with symptoms too mild to warrant operation should not be submitted to coronary arteriography as pointed out earlier. One debatable question is whether or not it is justifiable to submit patients to routine shunt angiography following aorto-coronary bypass. In the

present series there were 76 shunt studies most of which included repeat selective coronary arteriography as well.

The total complications of shunt angiography were one possible myocardial infarction and one local groin hematoma. With this very low incidence of complications it is presently felt that routine shunt studies should be done in order to evaluate the results of the bypass operation. When considering the risks of selective coronary arteriography it is appropriate to note that in the present material two patients with valvular disease and four with coronary disease scheduled for surgery died before their date of operation. This amounts to 2% of the total material which is higher than the combined mortality for selective coronary arteriography and aorto-coronary bypass operation in this hospital.

REFERENCES

1. Adams, D. P., Fraser, B. B. & Abrams, H. L. The complications of coronary arteriography. *Circulation* 48: 609 1973.
2. Grondin, C. M., Castonguay, Y. R., Lepage, G., Meier, C. & Grondin, P. Aortocoronary bypass grafts. *Arch. Surg.* 101: 535 1971.
3. Judkins, M. P. & Gander, M. P. Prevention of complications of coronary arteriography. *Circulation* 49: 599 1974.
4. McCarty, R. J. & Glasser, S. P. Thrombogenicity of guide wires. *Amer. J. Cardiol.* 32: 943 1973.
5. Morris, J. M., Chen, F. Y. & Rhenlander, H. F. Coronary hemodynamics following aorto-coronary bypass grafts. *Arch. Surg.* 103: 539 1971.
6. Morris, G. C., Reul, G. J., Howell, J. P., Crawford, E., S. Chapman, D. W., Beazley, H. L., Waters, W. L., Peterson, P. K. & Lewis, J. M. Follow-up results of distal coronary artery bypass for ischemic heart disease. *Amer. J. Cardiol.* 29: 180 1972.
7. Petch, M. C., Sutton, R. & Jefferson, K. E. Safety of coronary arteriography. *Brit. Heart J.* 33: 377 1973.
8. Takaro, T., Hultgren, H. N., Litzman, D. & Wright, E. C. An analysis of deaths occurring in association with coronary arteriography. *Amer. Heart J.* 86: 587 1973.
9. de la Torre, A., Jacobs, D., Aleman, J. & Anderua, G. A. Embolic coronary artery occlusion in percutaneous transluminal coronary arteriography. *Amer. Heart J.* 86: 467 1973.

AORTOCORONARY VEIN BYPASS IN PATIENTS WITH ANGINA PECTORIS

Bjørn Hoel, Harald Eie, Gudmund Semb and Egil Sivertsen

*From the University Institute for Respiratory Physiology, Department of Radiology,
Surgical Department III and Medical Department VIII
Ullevål Hospital, Oslo, Norway*

Abstract. In the 3-year period from May 1971 to April 1974 90 patients had aortocoronary bypass for angina pectoris at Ullevål Hospital. One patient died shortly after the operation (operative mortality 1.1%). There were no further deaths in the observation period. Clinical improvement was seen in 93% of the patients, early about pectoris in 92%. The study suggests that patients with isolated affection of the right coronary artery should not have bypass, because these patients 1) had less severe symptoms, 2) had better preserved left ventricular function, and 3) seemed to have a smaller chance of benefiting from the operation than the other patients. Multiple lesions gave good clinical results and carried no higher surgical risk than did single lesions. Good clinical results were seen also in patients with occluded branches provided they had at least one patent branch too. Graft occlusion occurred early and was associated with low graft flow as measured intraoperatively. Graft occlusion was not usually followed by demonstrable myocardial necrosis. In view of the small operative risk and the high score of symptom relief it is concluded that all patients with angina pectoris that does not readily respond to medical treatment, should be considered for aortocoronary bypass.

Since its introduction in 1967 (6) aortocoronary bypass has gained wide acceptance in the treatment of ischemic heart disease. This is largely due to the moderate and often dramatic relief of angina pectoris following the bypass operation. This improvement in the quality of life has been and still is the aim of this treatment.

The final position of the bypass operation in the management of coronary heart disease is, however, not yet defined. It will depend on long-term postoperative survival and infarction rates. Information about which is not available. The effect on short-term prognosis, i.e. operative and postoperative mortality and morbidity, is currently subject to much discussion, the outcome of which must also influence the future position of this treatment.

The purpose of this paper is to present a material of patients treated with aortocoronary vein bypass for angina pectoris which we think will demonstrate that the operative risk can be very small and the clinical results very good. The purpose is also to discuss some aspects of coronary artery disease in this material in relation to symptoms and results.

MATERIAL

The material consists of 90 patients, 77 men aged 28-65 years (mean 51) and 13 women aged 25-65 years (mean 51), who had aortocoronary vein bypass for angina pectoris during the 3-year period from May 1971 to April 1974 at Ullevål Hospital. The follow-up time was 1-37 months (mean 14). None of the patients had additional heart surgery. During the same period a number of other patients had bypass surgery plus valve repair or aortic aneurysmectomy. These patients will be presented in separate study and are not included in the present paper.

In 67 of the 90 patients the indication for bypass surgery was stable angina pectoris which medical treatment had failed to relieve satisfactorily. Twenty patients had unstable angina or imminent infarction. By unstable angina was understood the debut or marked aggravation of chest pain in the period prior to the operation. By imminent infarction was understood recurrent or long-lasting pain in hospital and/or transient ischemic changes in the ECG in patients admitted to hospital with acute coronary symptoms in whom the diagnosis of established acute myocardial infarction (AMI) was excluded. One patient was operated on for AMI. In two patients the indication for operation was coronary artery disease and serious arrhythmias resistant to medical therapy.

Patients above 65 years of age and patients with non-first left ventricular (LV) failure in spite of medical treatment were not operated on. AMI more than a few hours but less than 3 months old was another contraindication to operation. Hypertension, abnormal serum lipids, and previous myocardial infarction older than 3 months were not regarded as contraindications to surgery.

The vessels affected by significant coronary artery dis-

Table I Vessels affected

LAD=left anterior descending artery CP=circumflex artery RCA=right coronary artery

Vessels	No of pts.	No of vessels
LAD	8	Single vessel disease
CP	0	
RCA	8	
LAD+CP	11	Double vessel disease
LAD+RCA	20	
CP+RCA	6	
LAD+CP+RCA	37	Triple vessel disease

case, i.e. 30% stenosis of the lumen or more are shown in Table I. Most patients had double or triple vessel disease. The left anterior descending (LAD) artery was affected in 76 patients, the right coronary artery (RCA) in 71 patients, and the circumflex (CP) artery in 34 patients. Thus, affection of the CP artery was a little less common than affection of the other two main arteries in this material of operated patients.

In the 90 patients there was total of 133 grafts divided into 50 single, 35 double, and 5 triple grafts. Multiple grafts were separate in all but two cases, in whom a Y graft was used. The majority of the grafts were anastomosed to the LAD artery and to the RCA. End arterectomy was not done routinely but in 14 coronary arteries it proved necessary in order to obtain good distal graft to artery anastomosis or good distal run-off.

METHOD

All patients had bicycle exercise test, right heart catheterization, left ventriculography with recording of pressures before and after contrast and selective coronary angiography by Judkins technique preoperatively. The operation was done in moderate hypothermia with temporary clamping of the aorta and temporary cardiac standstill. The great saphenous vein was used for grafting. Coronary vessels with internal diameters down to 1 mm were accepted for distal anastomosis. Intraoperative graft flow was measured with an electromagnetic flowmeter. Anticoagulant therapy was started postoperatively and continued for about 3 months after the operation.

In most patients selective graft angiography was done 2-3 weeks after the operation, i.e. before the patients were discharged.

Follow-up angiographic and hemodynamic studies, several months postoperatively were only done in some patients, mostly those with residual symptoms. Late graft angiography and hemodynamics were therefore only obtained in these selected cases.

RESULTS

Complications

Of the 90 patients operated on, one has died (overall mortality 1.1%). He was a 48-year-old man with

chest pain at rest thought to have an imminent infarction. He died 5 hours after the operation. An autopsy showed that he had in fact an AMI about 48 hours old and he should therefore not have been operated on according to the criteria above.

The other complications are listed in Table II. There were 4 AMIs in connection with the operation (4.4%) and another 2 patients with the clinical picture of AMI but no definite ECG changes. Most of the AMI patients nevertheless showed clinical improvement following surgery (vide infra). All patients with postcardiotomy syndrome, wound infection and pericardial bleeding made a good recovery. There were no further deaths in the follow-up period but one patient had a small myocardial infarction 14 months after the operation and another patient had a possible infarction in the follow-up period.

A possible relationship between the number of shunts in each patient and the perioperative complications was looked into. In the 5 patients with triple shunts there were no serious complications. In the 35 patients with double shunts there were three manifest infarctions and in the 50 patients with single shunts there were one death due to myocardial infarction, one other manifest and two possible myocardial infarctions. Thus, there was no increase in complications in the patients who had multiple shunts.

Clinical results

The clinical results are based on the clinical assessment of the severity of angina pectoris before and after bypass surgery. Seventy-three patients

Table II Complications

Follow-up time 2-37 months (mean 14)

Complication	No of pts.	% of total pat. material
<i>Perioperative</i>		
Death	1	1.1
Manifest infarction	4	4.4
Possible infarction	2	2.2
Postcardiotomy syndrome	3 (+47)	3.3
Wound infection	3	3.3
Pericardial bleeding	3	3.3
<i>After discharge</i>		
Death	0	
Manifest infarction	1	1.1
Possible infarction	1	1.1

Table III. Correlation between complications and clinical results

Complication	Clinical results (no. of pts.)			
	Much Impr.	Im- proved	N change	Worse
<i>Preoperative</i>				
Death				1
Manifest infarction	3		1	
Possible infarction	2			
Postcardiotomy syn- drome	7			
Pericardial bleeding	2	1		
<i>After discharge</i>				
Manifest infarction		1		
Possible infarction		1		

(81.1%) were greatly improved and 10 patients (11.1%) were distinctly improved after the operation. Six patients (6.7%) did not benefit from surgery; their symptoms were unchanged but not worse. One patient (1.1%) died. Thus 93% of the patients were either much or distinctly improved after surgery.

Of 76 patients with a postoperative follow-up time of more than 3 months 63 patients (83%) returned to work. Five patients (7%) did not go back to work because of persistent angina pectoris. Another 8 patients (10%) did not return to work for other than cardiac. They were all clinically improved after the operation.

The clinical results were good even in patients perioperative and late complications. Table III shows the relationship between complications and clinical results.

The patient who was operated on for AMI was a 57-year-old man who arrived in hospital one hour after the onset of pain. ECG showed an extensive anterior infarction. A vein graft was put to his anterior descending artery 5 hours after admission. The immediate effect was a distinct reduction in the degree of ischemia surrounding the infarcted area. The patient made an uneventful recovery.

Shunt patency

The 135 shunts in the material 91 were examined by selective shunt angiography shortly after the operation (Fig. 1). Eighty-four shunts (92%) were patent and 7 (8%) were occluded. Thirty-one shunts

were examined at a late stage on an average 12 months postoperatively. Twenty-six shunts (84%) were patent and 5 (16%) were occluded. The total number of occluded shunts at early and late examination was 11 and the total number of patent shunts was 97. Mean flow in the patent shunts measured during the operation was 74 ml/min and in the occluded shunts 39 ml/min. One patient with a single shunt and another patient with two shunts had all shunts occluded. All the other patients had at least one shunt patent. Fourteen shunts were examined both early and late postoperatively. One was occluded on both occasions and 13 were patent on both occasions. Thus occlusion occurred early and there were no further occlusions between early and late angiography in these shunts.

Shunt occlusion was not usually followed by demonstrable myocardial infarction. On the contrary most infarctions occurred in patients with patent shunts. Thus, 3 of the 5 manifest and 2 of the 3 possible infarctions occurred in patients with all shunts patent. One manifest and one possible infarction occurred in two patients with one or more



Fig. 1 Shunt patency related to intraoperative shunt flow in 91 shunts examined early (1-4 weeks), and 31 examined late (average 12 months) postoperatively. O=patent shunts, @=occluded shunts, vertical line=shunt also examined early.

Table IV Correlation between vessels affected and LV function

PCV=pulmonary capillary venous pressure (during exercise) LVEDP=left ventricular end-diastolic pressure (after ventriculography) N patients had isolated CP affection

Vessels affected	Mean PCV (mmHg)	No of obs.	Mean LVEDP (mmHg)	No of obs.
<i>Single vessel disease</i>				
LAD	22.4	5	18.5	6
RCA	16.8	8	14.8	5
<i>Double vessel disease</i>				
LAD+CF	22.0	7	19.7	10
LAD+RCA	4.7	11	20.1	11
CF+RCA	4.1	6	21.4	5
<i>Triple vessel disease</i>				
LAD+CF+RCA	4.7	24	22.0	29

shunts occluded. In one patient with manifest infarction postoperative shunt angiography was not done

Hemodynamic results

Most patients had preoperative assessment of LV function by registration of pulmonary capillary venous pressure (PCV) during supine bicycle exercise and LV end-diastolic pressure (LVEDP) following left ventriculography. Table IV shows correlation between these parameters and extension and localization of significant coronary artery disease. PCV and LVEDP were elevated in all groups of patients except those with isolated affection of the RCA.

There was a limited number of comparable observation as regards LV function before and after the operation. Fig. 2 shows the effect of the operation on LVEDP following left ventriculography in 8 patients and on LV ejection fraction (LVEF) in 11 patients. LVEF was estimated from the left ventriculogram using the single plane area length method of Sandler and Dodge (13). LVEDP was reduced in 7 of 8 patients after the operation. A reduction was also seen in one patient with a single shunt that was found to be occluded. LVEF was increased in 9 of 12 patients after the operation, all 9 had patent shunts. Two of the three patients who showed a reduction in LVEF postoperatively had patent shunts while the third patient had a single shunt that was occluded. Of 15

patients with pre and postoperative left ventriculograms 7 had LV enlargement preoperatively. Only two of these showed a reduction in LV size postoperatively. In the remaining 5 patients, all of whom had patent shunts the LV enlargement persisted. One patient with a normal preoperative ventriculogram had an enlarged left ventricle postoperatively. He had a single shunt that remained patent, but he had a possible myocardial infarction in connection with the operation. In only 3 of 6 patients with patent shunts who had preoperative LV dyskinesia was LV contraction seen to be normal postoperatively. Shunt patency was therefore poorly related to improvement in LV dysfunction as expressed by LV enlargement or dyskinesia.

DISCUSSION

Indications for surgery

It is widely accepted that patients with stable disabling angina pectoris, resistant to medical treatment should be offered surgery. Most of the patients in our material fell into this category. It is our belief, however, in view of the good clinical results and the low mortality associated with the operation, that bypass surgery should be offered also to patients with somewhat less severe angina pectoris, provided the symptoms are severe enough to interfere with their daily activities. For the same reasons we think that patients with unstable angina

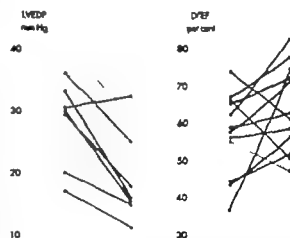


Fig. 2 LVEDP immediately following ventriculography in 8 patients before and after bypass surgery. LVEF in 11 patients before and after bypass surgery. — one patient with the only shunt occluded. The remainder had patent shunt

and imminent infarction should have the benefit of bypass surgery too. Most of these patients have in fact had previous stable angina which would make them candidates for surgery on the basis of symptom relief. The additional possibility of preventing an AMI should strengthen the indication for operation. In patients with angina pectoris of short duration, without previous symptoms it has been our policy however to delay surgery in the expectation of spontaneous improvement.

AMI is a special indication for aortocoronary bypass. In all reports the number of patients operated on is small, but most authors claim good results if the interval between the onset of symptoms and the operation does not exceed 5-6 hours (14) which is considered to be the limit for reversibility of myocardial ischemia. Our AMI patient demonstrated that the operation may be well tolerated and the area of permanent myocardial damage may be reduced.

The bypass operation carries a definite risk of serious complications and the selection of patients therefore has to be strict. A small mortality 1.1% in our material, must, however be accepted with this kind of treatment and weighed against the high score of symptomatic relief 93% in our material. It should be noted moreover that while there was one operative death 4 other patients in this hospital scheduled for bypass surgery died suddenly before the date of their operation, even though the waiting time for surgery was less than 6 weeks.

When discussing the complications of bypass treatment, one must also take into account the risks of the preoperative selective coronary arteriography and left ventriculography. So far there have been no fatal complications to preoperative angiography in patients investigated for coronary disease in this hospital (9) and no patients have been refused subsequent bypass surgery because of complications in relation to preoperative angiography.

Even in the presence of operative complications, most patients were improved clinically following surgery (Table III). Thus, 4 of 5 patients with definite myocardial infarction showed clinical improvement, and they all returned to work. This may possibly be explained by the fact that intraoperative infarctions tend to be small and of limited consequences. It also appeared that the postcardiotomy syndrome had no derogative effect on the final clinical result.

Single-double-triple vessel disease

In this material as in others most patients had double or triple vessel disease but 16 of the 90 patients operated on had lesions limited to a single main coronary vessel. Since these patients have a good prognosis of survival without surgery it is debatable whether they should be submitted to the risk of the operation. The operative mortality is so small, however especially in these patients with single vessel disease who are usually good risk patients that it should not be a major contraindication to surgery when the symptoms are severe, and the only efficient way of relief is by means of aortocoronary bypass. That patients with single vessel disease may have just as severe symptoms as patients with multiple vessel disease is demonstrated by the fact that in this material as many as 1/3 of the patients with single vessel disease had unstable angina or imminent infarction. To some extent this may be a reflection of patient selection because of patients with single vessel disease only those with severe symptoms were operated on. Patient selection was probably also the main explanation why there were no patients with isolated CF artery disease in this material since these patients, because of the technical difficulties associated with bypass to the CF artery would only be operated on in the presence of very severe symptoms.

There seemed to be a difference in severity of symptoms between the 8 patients who had isolated LAD involvement and the 8 who had isolated RCA involvement. In the former group, 4 of the 8 patients had unstable angina or imminent infarction, but in the latter group only one of the 8 had such severe symptoms. It is difficult to see why there should be such a difference unless it reflects a difference in collateral blood supply to the areas supplied by the two arteries.

If one looks at the relationship between arteries affected and clinical results in this material it is seen that in all 37 patients with triple artery involvement there was much improvement (34 pts) or improvement (3 pts.) following the operation. Likewise of the 37 patients with double artery involvement the majority showed much improvement (28 pts) or improvement (6 pts.) after the operation. Only 3 patients with combined LAD and RCA involvement were unchanged after the operation. In patients with single vessel disease however there was again a difference between patients with iso-

lated LAD disease and patients with isolated RCA disease. In the former group 7 of 8 patients were much improved and one patient was improved. In the latter one of the 8 patients died and another 3 were unchanged after the operation. Thus patients with isolated RCA disease only showed improvement in 50% of cases.

Number of shunts

Of the 40 patients who had double or triple shunts 33 patients were much improved and 6 were improved after the operation. Multiple shunts therefore gave good clinical results. Of the 50 patients with single shunts 6 failed to improve. It was concluded that multiple shunts gave a better chance of symptom relief than did single shunts and the risk of complications was no higher (*vide supra*).

Shunt patency

The great majority of the shunts studied by angiography postoperatively were patent. Of 55 patients who had all shunts patent at the early postoperative study 45 showed much clinical improvement and 6 showed improvement, whereas 4 patients had no change in symptoms after surgery. Thus about 7% of the patients did not benefit from the operation in spite of early postoperative shunt patency. Of the 79 patients with all shunts patent at late shunt angiography three (10%) failed to show clinical improvement. On the other hand, all three patients who had one of two shunts occluded at early shunt angiography nevertheless showed much clinical improvement, and one patient with two of three shunts closed was also much improved clinically as was one patient in whom the only shunt was occluded at late shunt angiography. There was therefore not a good correlation between shunt patency and clinical results. Determination of shunt patency must therefore rely on shunt angiography.

The discrepancy between postoperative symptoms and shunt patency is not difficult to explain. Even with all shunts patent there may still be areas of the myocardium that remain underperfused thereby maintaining the symptom of angina pectoris. On the other hand, relief of angina pectoris in patients with occluded shunts is seen when there are other shunts that remain open with a good collateral blood supply. The finding of all shunts occluded in the presence of good clinical

improvement as in one of our patients is less readily explained and a placebo effect cannot be excluded in such cases.

Shunt occlusion was directly related to shunt flow as measured intraoperatively. No shunts with a flow over 50 ml/min occluded. This is in agreement with other reports (10). The main factor determining shunt flow is the state of the peripheral vascular bed. It is clearly necessary therefore, to have a good distal run-off and patients with poor peripheral vessels should not be operated on. If the stenosis in the coronary vessel to be bypassed is only slight or moderate the coronary vessel may steal too much flow from the bypass graft and thereby cause graft occlusion. This was not felt to be the case in any of our patients as all bypassed vessels had at least a 50% reduction in diameter.

In our material all shunts that were patent at early shunt angiography remained patent also at late shunt angiography suggesting that shunt occlusion occurred early postoperatively if it occurred at all. Late shunt occlusion sometimes demonstrated to be the result of progressive fibrosis of the vein graft (4) the so-called graft disease was not observed in any of our patients.

Other reports have shown that shunt occlusion occurs in 5-15% of cases (5-11). In our material 16% of the shunts examined at a late stage were occluded. It must be pointed out however that late shunt angiography was mainly done in patients with residual symptoms and the occlusion frequency found may therefore be higher than in the material as a whole.

Most of the infarctions occurred in patients who had patent shunts. This supports the assumption that infarctions that do occur are located in areas not supplied by the coronary vessel operated on. Vice versa in the majority of patients with occluded shunts there were no demonstrable signs of myocardial infarction. It may be that in these patients the area supplied by the shunt was already fibrosed. Another possibility is that the area supplied by the shunt had a good collateral supply from other vessels. In each case the shunt would really be surplus and it would carry little blood which in turn would provoke early shunt occlusion. If these explanations for some cases of early shunt occlusion are accepted the problem clearly is one of preoperative assessment. So far our preoperative studies cannot with confidence tell us whether a particular area of the myocardium is

1) fibrotic beyond possibility of improvement through improved blood supply or 2) sufficiently perfused via collaterals from vessels other than the native vessel

Hemodynamics

It is interesting to look at the correlation between arteries affected by significant coronary disease and signs of LV failure preoperatively. Since patients operated on were not in manifest LV failure PCV during exercise and LVEDP following left ventriculography were chosen as indices to bring out any signs of latent LV failure. Table IV shows that the mean values for PCV and LVEDP for all groups were pathologically elevated, with the exception of patients with isolated RCA disease. It is concluded therefore that these patients with angina pectoris without clinical heart failure nevertheless had latent LV failure (its degree being independent of the extent of the coronary artery lesions as demonstrated by selective coronary arteriography). The possible exception to this was patients with isolated RCA disease, who seemed to have better preserved LV function. This is probably explained by the fact that the RCA contributes relatively little to the blood supply of the left ventricle.

Thus, it seems that coronary artery disease limited to the RCA was tolerated well by the patient both with respect to symptoms (vide supra) and with respect to LV function.

The good clinical results in this material are similar to those reported by others (7-11). It is assumed that relief of symptoms is mainly due to increased myocardial perfusion although definite proof of this is difficult to obtain. Attempts have been made to correlate symptom relief to improvement in LV function, which is thought to be a better indication of increased myocardial perfusion. It should be born in mind however that since these patients usually have normal or near normal LV function at rest preoperatively it may be difficult to demonstrate any improvement postoperatively unless the tests for LV function are made under some kind of stress. Some reports in the literature demonstrate no change in LV function following aortocoronary bypass although symptomatic relief from angina is good (1-6). Others claim improvement in LV function too (2, 12). Our observations did suggest some degree of improvement in LV function as expressed by LVEDP after left ventriculography and by LVEF in most of the patients who had compar-

able pre- and postoperative studies but the observations were too few to allow definite conclusions.

Limitations of the study

There are two main limitations in a study like the present. Firstly there is the subjectiveness on which the clinical results are based. Considering that the symptom of angina pectoris is quite readily influenced by a number of factors, many of which must undoubtedly have a placebo effect. It is an obvious drawback that the evaluation of the results should rest on the patients' experience of chest pain. In our opinion, however, having seen the patients before and after the bypass operation, it is quite clear that no placebo effect could be wholly responsible for the striking and uniform improvement in the clinical condition of most of these patients, the great majority of whom were able to return to full time work.

The second limitation lies in the lack of controls. In evaluating the effect of the bypass operation on the symptom of angina pectoris it is not practical to have a controlled study since this would require sternotomy and pericardiotomy in the randomized control patients too. Control as regards the effect on long-term survival is however quite possible but it would require a large number of patients and a long follow-up and falls outside the scope of this study.

ADDENDUM

Since the completion of this paper another 46 patients have been operated on with aortocoronary vein bypass for angina pectoris in this hospital. There have been no further deaths. This brings the total mortality of the operation down to less than 1%.

REFERENCES

1. Arbogast, R., Soligasc, A. & Boerama, M. G. Influence of aortocoronary saphenous vein bypass surgery on left ventricular volumes and ejection fraction. *Amer. J. Med.* 54: 290, 1973.
2. Chatterjee, E., Swan, H. J. C., Paroley, W. W., Sustala, H., Marcus, H. S. & Matloff, J. Influence of direct myocardial revascularization on left ventricular myography and function in patients with coronary heart disease. *Circulation* 47: 276, 1973.
3. Chesebrough, C., Effler, D. B., Loop, F. D., Groves, L. K., Sheldon, W. C., Razem, M. & Sonos, F. M. Emergency myocardial revascularization. *Amer. J. Cardiol.* 32: 901, 1973.
4. Dubost, C., Carpenter, A., Deloche, A., Soyfer, R. & Pownall, A. Direct coronary surgery. *J. cardiovasc. Surg.* 15: 149, 1974.

- 5 Effler D B, Favalaro R G & Groves L. K.. Myocardial revascularization. *J cardiovasc. Surg.* 12:1 1971
- 6 Favalaro R. G. Saphenous vein autograft replacement of severe segmental coronary artery occlusion. *Ann. thorac. Surg.* 5 334 1968.
- 7 Hall R. J, Dawson, J T, Cooley A. D, Hallman G L, Wukasch D C. & Garcia, E. Coronary artery bypass. *Circulation Suppl* III 146 1973
8. Hammermeister K E, Kennedy J W., Hamilton, G W., Stewart D h., Gould K. L., Lipscomb K. & Murray J A.. Aortocoronary saphenous-vein bypass. *New Engl J Med.* 290:186 1974
- 9 Hoel, B., Ele H., Semb G & Sivertsen E.. Selective coronary arteriography. *Acta med. scand* 197 377 1973
- 10 Moran, J M, Chen, P Y & Rheinlander H F. Coronary hemodynamics following aorto-coronary bypass graft. *Arch. Surg.* 101 539 1971
- 11 Morris, G C, Reul, G J., Howell J F, Crawford E. S, Chapman, D W, Beazley H. L., Winters, W L., Peterson P K. & Lewis, J M. Follow-up results of distal coronary artery bypass for ischemic heart disease. *Amer J Cardiol* 29:180 1972
12. Mordt, E. D, Harthorne J W, Buckley M J, Dismore R. & Auster, W G.. Direct coronary arterial revascularization. *Arch. Surg.* 101 529 1971
- 13 Sandler H & Dodge H T.. The use of single phase angiograms for the calculation of left ventricular volume in man. *Amer Heart J* 75 325 1968
- 14 Sustaita, H, Chatterjee K., Malloff J M, Marty A. T, Swan, H J C. & Fields, J.. Emergency bypass surgery in impending and complicated acute myocardial infarction. *Arch. Surg.* 105 30, 1972.

CARDIAC ARRHYTHMIAS ELECTROLYTES AND DIGOXIN CONCENTRATION IN PLASMA AND URINE IN PATIENTS TREATED WITH DIGOXIN

Åke Bertler Mario Monti Per Ohlin and Arne Redfors

From the Departments of Internal Medicine A, Clinical Physiology and the Division of Clinical Pharmacology University Hospital Lund S. eden

Abstract Cardiac arrhythmias, digoxin concentration in plasma and urine, digoxin and creatinine clearances, electrolytes in plasma and in erythrocytes, and subjective symptoms have been carefully studied for 3 consecutive days in 19 patients with definite or suspected digitalis intoxication. The digoxin treatment was discontinued during the observation period. Eleven controls without any signs of toxicity were similarly followed on unchanged maintenance dosage. All patients were independently classified as toxic or non-toxic from the follow-up of extended ECG recordings and subjective symptoms. In 9 definitely toxic patients plasma digoxin concentrations of 3.1 ± 0.7 ng/ml was found, as compared to 1.4 ± 0.5 ng/ml for the 11 controls. In the suspect toxic group $1.5-3.9$ ng/ml was found. The high digoxin level in the toxic group corresponds to a low digoxin clearance. In the toxic patients cardiac arrhythmias were related in most cases to a plasma digoxin level above 2.5 ng/ml and usually disappeared when the concentration had decreased below this. Suspect toxic patients, classified as probably non-toxic, and controls had with two exceptions plasma digoxin levels below 2 ng/ml. It is suggested that digitalis toxicity should be considered at plasma digoxin concentration above 2 ng/ml. It must be stressed that this level is not absolute and is affected by among other things, disturbance of intra- and extracellular electrolytes.

Despite long experience with the digitalis glycosides, the frequency of patients with symptoms of toxicity seems to have increased during recent decades. In studies on hospitalized patients treated with digitalis the reported incidence of toxicity has ranged from 7 to 23% (7).

Several reasons have been suggested for the increased incidence of digitalis toxicity. Major factors are the narrow therapeutic range of the glycosides

and an increased use of diuretics which disturb the electrolyte balance, the therapeutic dose at rapid digitalization, according to Lown and Le me (14) is about 63 % of the toxic dose. Similar values have been obtained for digoxin when given as maintenance treatment (15). Animal experiments have shown that approximately 50% of the lethal dose has been ingested when signs of digitalis toxicity appear.

A promising approach to these problems appeared when clinically useful methods—a radioimmunoassay (17) and a modified rubidium (^{86}Rb) method (16)—for direct estimates of plasma digoxin concentration became available. Several recent studies, using these techniques have shown significantly higher plasma digoxin levels in patients with signs of digitalis toxicity than in non-toxic patients treated with the glycoside. For a summary see Smith and Haber (18). In all investigations there is some overlap. In only two studies (11, 13) were the authors using a radioimmunoassay unable to differentiate between toxic and non-toxic patients.

A main problem is the difficulty of defining the toxicity of digitalis. ECG criteria often constitute the important basis for the diagnosis. However, a short routine ECG can give misleading results because rhythm disturbances may be of short duration, possibly intermittent, or unrelated to digitalis toxicity. The purpose of the present investigation was to study arrhythmias in patients treated with digoxin and to correlate them in the first place to plasma digoxin concentrations. Extended ECGs, extracardiac symptoms and plasma digoxin concentration measured with an ^{86}Rb method have been carefully registered over a period of 5 con-

Address reprint request to Dr Å. Bertler, Department of Clinical Pharmacology, The Medical School, S-58185 Linköping, Sweden.

Table 1 Clinical data on the patients

Pat. no.	Age (yr)	Sex	Diagnosis ^a	Extra-cardiac symptoms ^b	Diarr.	K subst.	Digoxin dosage (mg/d.)	Digoxin in plasma (ng/ml)	Digoxin clear (ml/min)	Creatin. clear (ml/min)
Toxic group										
1	79	♂	ASHD	ANV	+	+	0.25	3.2	77	54
2	79	♂	ASHD	-	-	-	0.375	2.6	43	NE
3	74	♀	ASHD+1+2	ANV	+	+	0.25	3.1	32	39
4	78	♂	HCVd	NVP	+	-	0.30	3.8	31	33
5	63	♀	RHD	A	-	-	0.25	3.2	20	51
6	77	♂	ASHD	-	+	+	0.125	3.8	10	8
7	89	♀	ASHD+3	ANV	+	+	0.25	3.9	39	30
8	71	♀	ASHD+4	AD	-	-	0.25	1.4	23	55
9	74	♀	HCVd+5	-	+	+	0.125	3.0	10	10
Suspect toxic group										
10	70	♂	ASHD+1+6	-	-	-	0.5	2.7	49	38
11	81	♂	ASHD	N	+	+	0.25	2.5	43	NE
12	81	♂	ASHD	NVD	+	+	0.375	1.7	66	NE
13	79	♂	ASHD+7	ANV	+	+	0.25	3.1	26	33
14	84	♀	ASHD	AD	+	+	0.25	2.6	29	37
15	62	♂	ASHD	-	+	-	0.375	2.2	42	60
16	76	♀	ASHD	ANV	+	+	0.375	3.9	42	42
17	84	♀	ASHD	-	+	+	0.25	2.6	44	42
18	51	♂	ASHD	P	+	+	0.375	1.5	89	86
19	67	♂	HCVd	ANV	+	+	0.375	2.7	14	9
Controls										
20	74	♂	ASHD+8	-	-	-	0.25	1.2	74	53
21	69	♂	ASHD+1+6	-	+	+	0.5	2.5	NE	40
22	69	♂	ASHD	-	-	-	0.5	0.7	108	69
23	62	♀	ASHD+1+3	-	+	-	0.25	1.2	60	57
24	85	♂	ASHD	-	+	+	0.5	2.1	32	81
25	77	♀	ASHD+3	-	+	-	0.31	1.0	60	83
26	63	♀	RHD	-	+	+	0.5	1.4	41	55
27	70	♂	ASHD	-	-	-	0.25	1.1	89	86
28	88	♀	ASHD+3	-	+	+	0.15	1.3	17	35
	71	♂	ASHD	-	+	+	0.25	1.6	25	78
	76	♂	ASHD	-	+	+	0.25	1.2	81	70

^a 1=diabetes mellitus, 2=cancer mammae c. met., 3=hyperthyroidism (treated), 4=hypertension, 5=pyelonephritis, 6=plasmocytoma, 7=lymphosarcoma, 8=cancer hepatis.

A=anorexia, N=nausea, V=vomiting, P=abdominal pain, D=diarrhoea.

Treatment with diuretics: + = treatment with furosemide in various dosage, except in case 18 in whom ethacrynic acid was used; in cases 21 and 23 complementary treatment with spironolactone was given.

Potassium substitution.

NE=not estimated.

secretion days. Furthermore creatinine and digoxin clearances were followed as well as some extra- and intracellular electrolytes.

DESIGN OF THE EXPERIMENT

The present investigation involved patients with various signs indicating digitalis intoxication, and a control material. The patients classified as toxic were mostly admitted because of their toxic symptoms, but some were already in the hospital when signs of intoxication developed. Patients who had taken their last digoxin dose more than 4 hours before admission were excluded, i.e. were those with acute myocardial infarction.

A preliminary classification of toxic or suspect toxic patients was performed by means of the case history and conventional preliminary 1-lead ECG registered shortly after admission. In these cases digoxin therapy was discontinued. The patients serving as matched controls were selected from the medical wards and were treated with their usual maintenance dose of digoxin throughout the investigation. They had no clinical or ECG signs of digitalis intoxication and went through the investigation as the other groups.

The case history was taken from a standardized printed questionnaire which enquired about symptoms that could be associated with digitalis toxicity: e.g. anorexia, nausea, vomiting, abdominal pain and diarrhoea. All patients were carefully followed in a standardized way for 5 days with

K ₀ (mEq/l)	K ₁ (mEq/l)	Na ₁ (mEq/l)	Electrolyte values diverging from normal range (mEq/l)
14	112	12.4	Cl/e 110
16	83	21.2	
16	93	11.1	Cl/e 88 Ca/e 5.5 Mg/e 1.4
17	117	23.6	
15	92	7.7	
15	101	14.0	
18	85	27.0	Na/ 128
18	79	11.1	
17	102	14.0	Na/e 137
15	84	26.4	Mg/e 1.5
15	89	12.4	Cl/e 97
14	105	19.4	
11	100	23.6	Cl/e 96 Ca/e 4.1
15	93	18.3	
15	106	10.4	
17	86	34.3	Cl/e 96
14	94	21.2	
18	91	17.3	Cl/e 97
16	89	21.2	
14	73	18.2	Cl/e 97 Na/e 136
16	102	9.3	Cl/e 97 Na/e 136
14	81	13.8	
17	90	13.2	Cl/e 111
17	84	29.4	Cl/e 111 Na/e 149
11	93	22.2	
13	101	15.6	
12	87	23.8	Mg/e 1.5
11	95	17.2	
12	88	16.8	
12	88	16.2	Mg/e 1.5

daily estimates of plasma digoxin, extra- and intracellular electrolytes, serum creatinine, and 24-hour creatinine and digoxin clearance. The first samples for estimating digoxin in plasma were drawn about 24 hours after the previous dose. In the control group the serial samples were always drawn 24 hours after the last dose given. Possible changes in the symptoms were specially asked for. A daily 12-lead ECG with a rhythm strip of 20 mm duration was included.

When patient had gone through the whole program, one of us (P. O.), who did not know anything about the patient, made an independent evaluation of the 20-min ECG. For the final, retrospective classification, the occurrence of cardiac arrhythmias and their disappearance when discontinuing the digitalis treatment were considered together with the clinical picture.

Criteria for definite and suspected digitalis intoxication

1. *Definite digitalis toxicity* (A) Marked extracardiac symptoms which could be related to digitalis toxicity (B) Certain rhythm disturbances: paroxysmal atrial tachycardia with atrioventricular block, atrioventricular nodal escape rhythm, multifocal ventricular extrasystoles, ventricular bigeminy or trigeminy 2nd degree AV block. For the diagnosis, B in most cases combined with A, should be present and should disappear when digitalis treatment is discontinued.

2. *Suspected digitalis toxicity* (A) Extracardiac symptoms which could be related to digitalis toxicity (B) Certain rhythm disturbances: sinus bradycardia, sinoatrial block. For the diagnosis, A or B should be present and should disappear when digitalis treatment is discontinued, or arrhythmia under 1 B should be present but not disappear completely when digitalis treatment is discontinued. Also patients preliminarily classified as toxic or suspected toxic are included.

MATERIAL

Most of the patients with suspected or definite intoxication, according to the final classification, had been treated with an unchanged digoxin dose for more than one month, often for many years. One patient classified as suspected intoxication, had received the glycoside for only 8 days. The patients serving as controls had similarly been treated for a long time with an unchanged digoxin dose, except for two who had received the glycoside for only 8 and 9 days, respectively.

The digoxin dosage was 0.125–0.375 mg once a day except for one toxic patient, who received 0.5 mg daily. All patients were treated with the same brand of digoxin (Lanacrist® Draco Lund, subsidiary to Astra, Sweden).

According to the final classification, 9 patients belonged to the toxic group, 10 to the suspect toxic group and 11 were accepted as controls. The first two groups comprised 8 females and 11 males; their ages ranged from 51 to 89 years (mean 74). The control group contained 4 females and 7 males; their ages varying from 51 to 88 years (mean 73).

The indication for digoxin treatment in most cases was congestive heart failure. Two of the controls were given digoxin for atrial fibrillation alone. In the group of definite and suspected intoxication, 15 patients had arteriosclerotic heart disease (ASHD), in one case combined with therapeutically corrected hyperthyroidism, 3 had hypertensive cardiovascular disease (HCV), and one had rheumatic heart disease (RHD). In the control group 10 patients had ASHD. In three of them combined with treated hyperthyroidism without signs of activity and one had RHD. Further details about the patients are given in Table 1.

METHODS

Digoxin concentration in plasma

The method used is a modification of the ¹²⁵Ib method previously described in detail (4). For an analysis, 2 ml of plasma was extracted with 5 ml of newly distilled di-

chloromethane. After 10 min of centrifugation at about $1\,400\times g$ the plasma was soaked off and 4 ml of the clear dichloromethane phase was transferred to new tubes. These were put into a water bath and placed in a flame cupboard. After about 30 min at 50°C , the temperature was increased to 70°C to ensure a complete evaporation of the extracts. The residue was then dissolved in 1 ml of NaCl glucose solution, and 0.5 ml of washed packed red cells was added to each sample. This was followed by a preincubation in a water bath at 37°C for 2 hours. After cooling the samples in ice water for a few minutes, about 6 μCi of ^{86}Rb was added and the incubation at 37°C continued for another hour. Thereafter the samples were centrifuged. The supernatant was soaked off and the cells were washed twice with about 10 ml of saline solution.

Finally the γ -activity of the washed and packed erythrocytes was registered in a well crystal. In each analysis, standards containing known amounts of digoxin and samples without digoxin were run parallel with the patient samples, performed in triplicate.

The S.D. of the method is 0.1 ng digoxin/ml plasma within the triplicate determinations as well as between these triplicates performed on different days.

Digoxin concentration in urine

The procedure for urine assay was mainly as described for plasma samples. In the standard series including zero samples, however, normal urine, containing no digoxin or other heart glycosides, was used instead of glycoside-free plasma. As the digoxin content in patient urine was mostly rather high, a screening assay was always made in order to choose convenient volume for each sample, not consisting more than 6 ng digoxin. This was done because the standard reference curve could be regarded as straight only up to a concentration of 3 ng/ml. In most cases 50 μl of urine was used for the assay. This volume was diluted up to 2 ml with normal urine thereafter the assay was run identically as for plasma.

RESULTS

The amount of digoxin excreted daily in urine during days was estimated. In toxic and suspect toxic patients, the mean plasma digoxin concentration of the separate days was known and the 24-hour digoxin clearance was calculated. In the control patients, the daily fluctuation in plasma levels after dose administration was taken into consideration. Planimetric calculations showed that a value 0.3 ng/ml higher than the mean of the plasma concentrations enclosing the 24-hour period should be used.

Creatinine was estimated by the method described by Jaffe and modified by Henry (14), eliminating non-creatinine chromogens. Values given in Table I for both digoxin and creatinine clearances are the mean of repeated 24-hour clearances.

Electrolytes in red blood cells and in plasma

A direct method, described by Bengtsson et al. (3), was used for the determination of electrolytes in erythrocytes. Blood samples of 5–8 ml were drawn from the antecubital fossa, in most cases without stasis. In a few patients a slight stasis was applied during the venous puncture thereafter blood was drawn without stasis. Coagulation

was inhibited by sodium heparin. The blood was immediately centrifuged at $2\,000\times g$ for 3 min, plasma was then removed. The erythrocytes were washed twice in an equal volume of choline Ringer solution (choline chloride 146 MgCl₂ 1 CaCl₂ 1 NaCl 5 orthophosphoric acid 2.5 mM tris buffer to pH 7.4). The erythrocyte suspension was centrifuged at $2\,000\times g$ for 3 min. After each washing the choline Ringer solution was removed. The erythrocytes were haemolyzed and diluted with distilled water. For sodium determination 5 ml distilled water was added to 100 μl erythrocytes. For potassium, the samples were further diluted 1:11. Each sample was analysed four times in a Unicam SP 90 flame photometer. All figures were corrected for trapped extracellular fluid and for water lost during washing and centrifugation according to Bengtsson et al. (3).

The S.D. of the method, within four determinations on the same material, was 0.3 mEq sodium and 2.1 mEq potassium per 1 RBC. The S.D. of determinations made on different days gave similar results, 0.3 mEq sodium and 2.4 mEq potassium respectively.

The range of intracellular sodium concentration (Na/i) in 30 normal subjects (15 males and 15 females) has been found to be 5.7–10.3 mEq/l of RBC (mean 8.0). The range of intracellular potassium concentration (K/i) was 84–112 mEq/l of RBC (mean 98).

Sodium, potassium, and calcium in serum were determined with flame photometry. For estimation of magnesium in serum, a spectrophotofluorometric method was used.

Electrocardiography

A preliminary conventional 12-lead ECG was registered at the beginning of the investigation. As a routine this ECG was registered for 10 sec in patients with regular rhythm and for 40 sec in patients with arrhythmias. The information from the ECG was considered in the preliminary classification of the patients.

The patients were followed up for 3 days with a daily 20-min ECG, registered continuously with the patients in complete bed rest. The ventricular rate was estimated by counting all the QRS complexes during the last minute of registration.

RESULTS

Plasma digoxin concentration and urinary excretion of digoxin

No correlation was found between the dose administered and the plasma digoxin levels registered. Most of the patients 19 of 30 had been treated with 0.25 mg once daily. They showed plasma digoxin levels ranging from 0.7 to 3.9 ng/ml that correlated significantly ($p < 0.01$) with the digoxin clearance. In the group of definite intoxications 2 of 9 patients had only received 0.1–0.5 mg digoxin daily. Despite the low dose their plasma levels were 3.8 and 3.0 ng/ml respectively.

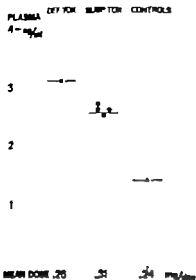


Fig. 1 Plasma digoxin levels and the mean digoxin dosage in the definitely toxic, suspect toxic, and control patients.

Patients with definite digitalis intoxication had with one exception, high plasma digoxin levels 2.6–3.9 ng/ml 24 hours after the last dose (Table I, Fig. 1). Most controls had plasma values well below 2 ng/ml (mean 1.4). Only two of them showed values above 2 ng/ml. The patients with suspected digitalis intoxication had plasma digoxin levels ranging from 1.5 to 3.9 ng/ml.

The control patients receiving an unchanged digoxin maintenance dose during the 5 days showed no significant change in mean plasma

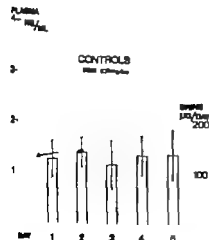


Fig. 2 Mean plasma digoxin levels (—) and urinary excretion of digoxin for the 5 days of observation in the control patients (mean \pm S.D.).

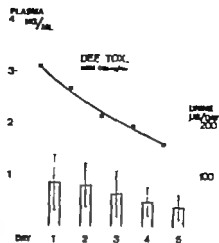


Fig. 3 Mean plasma digoxin levels (—) and urinary excretion of digoxin for the 5 days of observation in the patients with definite digitalis toxicity (mean \pm S.D.).

digoxin levels during the study (Fig. 2). The weighted S.D. between examinations was 0.2 ng/ml. In the other patient groups plasma digoxin concentration declined about linearly when the digoxin therapy was discontinued (Figs. 3 and 4).

In the control patients a mean of 129 μ g digoxin was found daily in the urine (Fig. 2) which is 54% of the average maintenance digoxin dose. Great variations were seen between the patients, ranging from 28 to 70% of the daily dose. No significant difference was found in urinary digoxin excretion

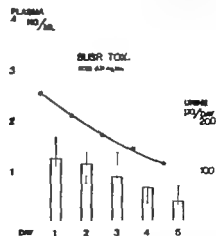


Fig. 4 Mean plasma digoxin levels (—) and urinary excretion of digoxin for the 5 days of observation in the patients with suspected digitalis toxicity (mean \pm S.D.).

Table II. Cardiac arrhythmias and their correlation to plasma digoxin levels

Digoxin/Pl. at App = plasma digoxin level when an arrhythmia was observed initially. When two values are given, the arrhythmia did not disappear. In these cases, the highest and lowest digoxin values registered are given. Disapp = plasma digoxin level when an arrhythmia disappeared.

Type of arrhythmia	Definite toxic		Digoxin/Pl. at		Suspect toxic		Digoxin/Pl. at	
	Case no.	Disappeared in case no	App.	Disapp.	Case no	Disappeared in case no	App.	Disapp.
PAT with block	6	6	3.8	4.0 ^a				
AV nodal escape rhythm	2		2.6	1.5				
	4	4	3.8	3.6 ^b				
	1	1	3.9	.3				
Ventricular bigeminy or trigeminy	3	3	3.2	2.1				
	5	5	3.1	1.6				
	7	7	3.2	1.8				
Multifocal ventr. extrasystoles	9	9	3.0	2.5	12	-	1.7-2.7	
					18		1.5-0.7	
2nd degree AV block	8	8	1.4	0.9				
Sinus bradycardia					19	19	2.7	1.8
SA block					11	11	2.5	1.4
Sinus arrhythmia	1	-	3.1-1.5		10	-	2.7-1.2	
Atrial extrasystoles	1	-	3.2-1.5		10	-	2.7-1.2	
	3		3.1-1.6		13		3.1-2	
	6		4.0-2.0		17		2.6-1.4	
	8		1.4-0.7					
Unifocal ventr. extrasystoles (<5/min)	4	-	3.8-1.3		10		2.7-1.2	
					11	-	2.5-1.2	
					14		2.6-1.1	
					16		3.9-2.5	
					17		2.6-1.1	

A 1st degree AV block persisted.

A atrial fibrillation with a ventricular rate below 50/min persisted.

from one day to another. In the toxic and suspect toxic patient group the total daily amount of digoxin found in the urine fell continuously during the observation period (Figs. 3 and 4).

The data for these groups are not comparable with those for the control patients who were still on digitalis treatment.

Renal function was impaired in many of these old patients, especially in the toxic group (Table I). There was a good correlation between creatinine and digoxin clearances ($r=0.83$, $p<0.001$) (Fig. 5). However, discrepancies were noted in some patients. The digoxin clearance for the toxic group was 26 ± 12 ml/min and for the controls 39 ± 30 ml/min, the suspect toxic group was intermediate 44 ± 21 ml/min. The difference between the toxic and control patients is significant ($p<0.01$).

Electrocardiographic findings

The basic rhythm was found to be sinus rhythm in 6 of 11 patients in the control group; the remaining

controls had atrial fibrillation. In the toxic and suspect toxic group sinus rhythm was observed in 9 patients; nodal rhythm in one and atrial fibrillation in 9.

In the toxic group the following rhythm disturbances were seen (Table II): paroxysmal atrial tachycardia with AV block—PAT with block (1 patient); AV nodal escape rhythm (2 patients); second degree AV block (1 patient); ventricular bigeminy or trigeminy (4 patients) and multifocal ventricular extrasystoles (1 patient). In the cases of bigeminy or trigeminy the ventricular extrasystoles were unifocal in 3 patients and multifocal in one. In two patients (cases 4 and 6) the arrhythmias (AV nodal escape rhythm and PAT with block) disappeared as early as on the second day of observation despite persistently high plasma digoxin levels (3.6 and 4 ng/ml, respectively) but these arrhythmias were followed by an atrial fibrillation with a ventricular rate below 50/min (case 4) and a 1st degree AV block (case 6). These two changes were both considered

Controls			
Case no.	Disappeared because no	Digoxin/Pl. at	
		App.	Disapp.
2	30	2.3-1.8 1.5-1.3 0.8	1.3
3	-	2.7-2.2 1.1-0.9 1.6-1.3 1.3 1.6	

to be digitalis-induced as the bradycardia disappeared and the PR intervals gradually shortened during the following days with decreasing plasma digoxin levels.

All the three patient groups displayed sinus arrhythmia, atrial extrasystoles and unifocal ventricular extrasystoles (less than 5/min). These rhythm disturbances were seen within a wide range of plasma digoxin levels and they did not change significantly in the toxic and suspect groups when the digitalis therapy was discontinued. Therefore they were considered to be unrelated to digitalis toxicity.

Ten patients belonged to the group of suspected digitalis intoxication. One of these had sinoatrial block (SA block) and one had sinus bradycardia (heart rate below 50/min). These two rhythm disturbances disappeared when digitalis was discontinued. Six patients belonging to the group showed no arrhythmia which could be attributed to digitalis toxicity. In two other patients, multifocal ventricu-

lar extrasystoles were observed. However they did not disappear or change significantly in frequency when digitalis was discontinued, even though the plasma digoxin fell to low values (0.7 ng/ml). Therefore it was concluded that the multifocal ventricular extrasystoles in these two cases were not related to digitalis intoxication. In the 11 controls no arrhythmia attributable to digitalis toxicity was observed (Table II).

The details regarding the relation between cardiac arrhythmias and plasma digoxin levels are given in Table II.

Extra- and intracellular electrolyte concentrations

In the control patients, the mean concentration of Na⁺ for 5 days was found to range from 9.3 to 29.4 mEq/l with a mean of 17.8 mEq/l. For the suspect toxic and toxic groups the initial means were 20.4 (range 10.4-34.3) and 15.6 (range 7.7-27.0) mEq/l.

The range of K⁺ in the control group was 75-102 mEq/l (mean 90.5). For the suspect toxic group the initial mean was 93.3 (range 84-106) mEq/l. For the toxic group it was 95.8 (range 79-117) mEq/l.

The extracellular sodium concentration (Na⁺) ranged from 128 to 149 mEq/l (mean 140.4). There was practically no difference between the means for the three groups. In the control group 2 patients had a slight hyponatraemia (136 mEq/l) and one a slight hypernatraemia (149 mEq/l). In the group of

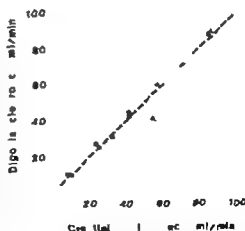


Fig. 5 The correlation between digoxin and creatinine clearances in definitely toxic (\bullet), suspect toxic (\circ), and control (Δ) patients ($r=0.83$).

digitalis intoxications, 137 mEq/l was registered in one patient and 128 mEq/l in another. Hypopotasæmia (3.2 mEq/l) was found in one patient in the control group. Increased extracellular serum potassium (K/e) 5.1 and 5.4 mEq/l was found in 2 patients in the control group and in 3 in the suspect toxic group (5.6, 5.4 and 5.0 mEq/l). Mean K/e was somewhat lower in the toxic group than in the others ($p < 0.05$) but still well within normal limits. Extracellular serum calcium (Ca/e) was slightly increased (5.5 mEq/l) in one patient in the toxic group and slightly decreased (4.1 mEq/l) in one subject in the suspect toxic group. Extracellular serum chloride (Cl/e) was slightly elevated (111 mEq/l) in one control patient and slightly decreased in 7 patients. Extracellular magnesium (Mg/e) was slightly decreased in 4 patients (1.4–1.5 mEq/l), otherwise normal.

The intra- and extracellular electrolyte concentrations showed no systematic changes during 5 days of observation.

DISCUSSION

In the evaluation of plasma digoxin as a tool in the diagnosis of digitalis intoxication the criteria for toxicity are evidently of decisive importance. In previous investigations, the classification of digitalis toxicity has been based largely on ECG findings. Unfortunately there is no cardiac arrhythmia specific for digitalis toxicity. Furthermore a transitory arrhythmia, possibly related to digitalis toxicity may be missed in the registration of only short conventional routine ECGs or rhythm strips. At the same time such an arrhythmia can be judged erroneously as "disappearing" if it is missing in a subsequent short serial recording. Therefore in the present investigation, the ECGs were registered 20 min daily for 5 days.

Even if carefully studied cardiac arrhythmias might not be sufficient to determine the diagnosis of digitalis toxicity. Pronounced subjective symptoms indicating toxicity were therefore taken into account as well. These symptoms must be appraised in relation to the whole clinical picture.

Because ECG findings play such an important role for the classification of digitalis toxicity it is of particular interest to correlate rhythm disturbances and their possible disappearance to the plasma digoxin levels (Table II). PAT with block, AV nodal escape rhythm, ventricular bigeminy or

trigeminy and multifocal ventricular extrasystoles were observed in 8 patients and were considered to be related to digitalis toxicity. When these arrhythmias were observed initially the plasma digoxin level was more than 2.5 ng/ml in all 8 patients. When they disappeared the plasma digoxin concentrations were 2.5 ng/ml or less in 6 of the 8 patients. In the remaining two patients other manifestations of digitalis toxicity persisted until the digoxin level had decreased (Table II).

The eight toxic patients discussed so far had plasma digoxin levels well above 2.0 ng/ml. Case 8 however classified as toxic because of a 2nd degree AV block and extracardiac symptoms which disappeared when digitalis therapy was discontinued had a plasma digoxin concentration usually regarded as non-toxic (1.4 ng/ml). It is interesting to note that, despite a normal K/e value she had low intracellular concentrations of this ion (Table I). Among the extracardiac symptoms, this patient suffered from a marked diarrhoea that had started about one week before admission in hospital. It seems probable that this was an important reason for her electrolyte disturbance triggering toxic cardiac symptoms at a non-toxic plasma digoxin level.

Ten patients had rhythm disturbances and extracardiac symptoms which placed them in the group of suspected digitalis toxicity. Two of these patients might be regarded as toxic since their rhythm disturbances—AV block (case 11) and sinus bradycardia with a ventricular rate below 50/min (case 19)—disappeared when digitalis therapy was discontinued (Table II). Another two of these patients (cases 12 and 18) were probably non-toxic because their arrhythmia (multifocal ventricular extrasystoles) persisted although they received no digitalis. It is of particular interest that the plasma digoxin level was above 2 ng/ml in the two patients considered to be toxic and well below this level in those considered non-toxic. Thus the digitalis estimate agrees with and strengthens the clinical evaluation. Six of 10 patients in the suspect toxic group cannot clinically be further divided into toxic or non-toxic since their extracardiac symptoms and rhythm disturbances did not change significantly although the digitalis therapy was discontinued. In two of them (cases 13 and 16) this might be because that even 5 days was too short an observation period. Their plasma levels on admission were

3 and 3.9 ng/ml respectively and had decreased to 2.2 and 2.5 ng/ml respectively by the last day. They might thus have been toxic despite persistent symptoms and arrhythmias. In the remaining 4 patients the plasma levels were on the whole intermediate between those registered in the toxic group and in the controls (2.1–2.7 ng/ml).

Multifocal ventricular extrasystoles are of particular interest since they were observed in 3 patients at a plasma digoxin level of 3.0, 1.7 and 1.5 ng/ml respectively (see above). The multifocal ventricular extrasystoles disappeared in only the first of these three patients. In the other two the ectopic beats remained for the 5 days although the plasma digoxin level had decreased to 0.7 ng/ml. It seems reasonable to conclude that multifocal extrasystoles are an uncertain indicator of digitalis toxicity. Fogelman et al. (11) who found no correlation between plasma digoxin level and digitalis toxicity seem to have based the diagnosis of digitalis toxicity on multifocal ventricular extrasystoles in 5 of 18 cases, which seems to be dangerous.

The plasma digoxin estimate seems to be of value in the diagnosis of digitalis intoxication. The present data indicate that digitalis intoxication is highly probable when the plasma digoxin level is above 3 ng/ml. Furthermore a plasma digoxin level below 2 ng/ml can be considered as non-toxic in patients with normal electrolyte values. According to our experience plasma digoxin levels above 2 ng/ml should arouse suspicion of digitalis intoxication even in the absence of clinical signs of toxicity. These patients should be carefully controlled and a reduction of their digoxin dosage should be considered as exemplified by one control patient (case 21). During the 5 days he had a mean plasma digoxin level of 2.5 ng/ml. On unchanged digoxin dosage he developed a definite digitalis intoxication a few weeks later.

The electrolyte balance is one of the factors influencing a patient's sensitivity to digitalis glycosides. It is well known that digitalis preparations have an inhibitory effect on the active cation transport through e.g. the red cell membrane. In the present material 26 patients (87%) were found to have abnormally high concentrations of intracellular sodium (Table I). Our results agree with those of Tosa (19) who found that 88% of a group of rapidly digitalized patients had increased Na/I concentrations. Hypertension may have contributed to the

intracellular hypernatremia in some of the patients this condition being capable of inducing a high sodium concentration in red blood cells. No significant correlation was found between digoxin concentration in plasma and sodium concentration in RBC.

Tosa (19) found decreased K/I concentration in 78% of digitalized patients. In our material only 6 patients of 30 (20%) were found to have decreased potassium concentrations in RBC. The lower incidence of hypokalaemia as well as the lack of correlation between the plasma digoxin concentration and K/I in our material might be due to a generous potassium substitution. No significant correlation was found between sodium and potassium concentrations in the RBC.

Digoxin is primarily excreted unchanged in the urine. Bloom and Nelp (8) and later Doherty and Flanigan (10) using tritiated glycoside, showed that digoxin clearance corresponded well to the creatinine clearance. This was confirmed in the present study (Fig. 5). Contrary to our findings a poor correlation between the two clearances has been reported recently with digoxin clearances consistently lower than those of creatinine. The discrepancy between our results and those of Bayliss et al. (1) probably has methodological reasons. The radioimmunoassay used by these authors was shown recently to be influenced by substances other than digoxin (7). In a study on the reliability of radioimmunoassay for direct estimates of urinary digoxin concentration, we found that urine from subjects not treated with digoxin or other heart glycosides might contain more than 6 ng "digoxin units"/ml. Hormone metabolites with a steroid nucleus are probably the reason for these erroneously elevated values. The ^{86}Rb method yields reliable urine glycoside values.

The daily excretion of digoxin fell after digoxin treatment had been discontinued in the patients with definite or suspected digitalis toxicity. In the definite toxic group the mean urinary excretion was somewhat less than in the group of suspected toxicity despite higher plasma digoxin values probably due to a more impaired renal function (Table I).

The control patients excreted in the urine on an average 54% of the daily maintenance digoxin dose. There were large interindividual differences—28–70% of the digoxin dose ingested—in most cases correlated to the renal function. It

digitalis intoxications 137 mEq/l was registered in one patient and 128 mEq/l in another. Hypopotassemia (3... mEq/l) was found in one patient in the control group. Increased extracellular serum potassium (K/e) 5.1 and 5.4 mEq/l was found in 2 patients in the control group and in 3 in the suspect toxic group (5.6, 5.4 and 5.0 mEq/l). Mean K/e was somewhat lower in the toxic group than in the others ($p < 0.05$) but still well within normal limits. Extracellular serum calcium (Ca/e) was slightly increased (3.5 mEq/l) in one patient in the toxic group and slightly decreased (4.1 mEq/l) in one subject in the suspect toxic group. Extracellular serum chloride (Cl/e) was slightly elevated (111 mEq/l) in one control patient and slightly decreased in 7 patients. Extracellular magnesium (Mg/e) was slightly decreased in 4 patients (1.4–1.5 mEq/l) otherwise normal.

The intra- and extracellular electrolyte concentrations showed no systematic changes during 5 days of observation.

DISCUSSION

In the evaluation of plasma digoxin as a tool in the diagnosis of digitalis intoxication, the criteria for toxicity are evidently of decisive importance. In previous investigations, the classification of digitalis toxicity has been based largely on ECG findings. Unfortunately there is no cardiac arrhythmia specific for digitalis toxicity. Furthermore a 'transitory arrhythmia, possibly related to digitalis toxicity' may be missed in the registration of only short conventional routine ECGs or thin strips. At the same time such an arrhythmia can be judged erroneously as "disappearing" if it is missing in a subsequent short serial recording. Therefore in the present investigation, the ECGs were registered 70 min daily for 5 days.

Even if carefully studied cardiac arrhythmias might not be sufficient to determine the diagnosis of digitalis toxicity. Pronounced subjective symptoms indicating toxicity were therefore taken into account as well. These symptoms must be appraised in relation to the whole clinical picture.

Because ECG findings play such an important role for the classification of digitalis toxicity it is of particular interest to correlate rhythm disturbances and their possible disappearance to the plasma digoxin levels (Table II). PAT with block, AV nodal escape rhythm, ventricular bigeminy or

trigeminy and multifocal ventricular extrasystoles were observed in 8 patients and were considered to be related to digitalis toxicity. When these arrhythmias were observed initially the plasma digoxin level was more than 2.5 ng/ml in all 8 patients. When they disappeared the plasma digoxin concentrations were 2.5 ng/ml or less in 6 of the 8 patients. In the remaining two patients other manifestations of digitalis toxicity persisted until the digoxin level had decreased (Table II).

The eight toxic patients discussed so far had plasma digoxin levels well above 2.0 ng/ml. Case 8 however classified as toxic because of a 2nd degree AV block and extracardiac symptoms which disappeared when digitalis therapy was discontinued, had a plasma digoxin concentration usually regarded as non-toxic (1.4 ng/ml). It is interesting to note that despite a normal K/e value, she had low intracellular concentrations of this ion (Table I). Among the extracardiac symptoms, this patient suffered from a marked diarrhoea that had started about one week before admission to hospital. It seems probable that this was an important reason for her electrolyte disturbance triggering toxic cardiac symptoms at a non-toxic plasma digoxin level.

Ten patients had rhythm disturbances and extracardiac symptoms which placed them in the group of suspected digitalis toxicity. Two of these patients might be regarded as toxic since their rhythm disturbances—AV block (case 11) and sinus bradycardia with a ventricular rate below 50/min (case 19)—disappeared when digitalis therapy was discontinued (Table II). Another two of these patients (cases 12 and 18) were probably non-toxic, because their arrhythmia (multifocal ventricular extrasystoles) persisted although they received no digitalis. It is of particular interest that the plasma digoxin level was above 2 ng/ml in the two patients considered to be toxic and well below this level in those considered non-toxic. Thus the digitalis estimate agrees with and strengthens the clinical evaluation. Six of 10 patients in the suspect toxic group cannot clinically be further divided into toxic or non-toxic since their extracardiac symptoms and rhythm disturbances did not change significantly although the digitalis therapy was discontinued. In two of them (cases 13 and 16) this might be because that even 5 days was too short an observation period. Their plasma levels on admission were

16. Redfors, A. & Bertler, Å. Estimation of plasma digoxin and its diagnostic use in suspected digitalis induced arrhythmias. In: *Symp on Cardiac Arrhythmias* (ed. E. Sandae), pp. 601-612. Astra, Elsinore 1970.
17. Smith, T. W., Butler, V. B. & Haber, E. Determination of therapeutic and toxic serum digoxin concentrations by radioimmunoassay. *New Engl. J. Med.* 281: 1212, 1969.
18. Smith, T. W. & Haber, E.. Digitalis. *New Engl. J. Med.* 289: 1063, 1973.
19. Tosa, M. Cardiac glycoside and electrolytes in cardiac insufficiency. *Kobe J. Med. Sci.* 16: 1, 1970.

REGISTRATION OF SINUS NODE RECOVERY TIME IN PATIENTS WITH SINUS RHYTHM AND IN PATIENTS WITH DYSRHYTHMIAS

Helge Grendahl Målfrid Müller and Egil Sivertsen

From Medical Department VIII Ullevål Hospital Oslo Norway

Abstract. Sinus node recovery time (SRT) after rapid atrial pacing has been recorded in 66 patients: 28 with coronary heart disease, 11 with advanced AV block, 10 with sick sinus syndrome and 17 with paroxysmal tachycardic rhythm. In patients with normal functioning sinus node SRT was related to the basal heart rate. On an average SRT was 130% of the basal P-P interval with an upper limit of 160%. In patients with presumed normal atrial function the mean SRT was found to be 1080 msec, with an upper limit of 1300 msec. This corresponds with previously published observations. In all 5 patients examined, β -receptor blockade (propranolol 5 mg l.) prolonged SRT. The prolonged SRT was related to sinus bradycardia. Verapamil (Isoptin® 5 mg l.) had no effect of SRT in the 7 patients examined. The observation of an SRT of more than 1300 msec indicates a poor sinus node function. Recording of a normal SRT however cannot exclude a sinus node dysfunction, as normal SRT is occasionally found even in patients with a clinically proved dysfunction.

The clinical manifestations of sinus node dysfunction include sinoatrial block, sinus arrest, sinus bradycardia and tachybradycardia syndrome. In patients in whom these disturbances are recorded or suspected a complete evaluation of the sinus node function is warranted. Methods for examination of the sinus node function are at present insufficient. It has not been possible to record either ECGs from the sinus node in patients or the sinoatrial transmission time. The sinus node automaticity however can be tested by recording the postpacing suppression. This interval is called the sinus node recovery time (SRT). SRT can be recorded after induction of a supraventricular extrasystole by atrial pacing (4) or more conveniently after abrupt cessation of atrial overdrive pacing (3, 4, 6).

The purpose of the present study has been to record SRT in patients with a presumably normal

sinus node function, and in patients with clinical signs of sinus node dysfunction.

METHODS

Atrial pacing was performed with bipolar pacing electrodes introduced percutaneously from the right cubital or the femoral vein. The pacing electrodes were placed adjacent to the sinus node in the upper posterolateral part of the right atrium. Usually two pacing catheters were introduced, and intratrial P waves were recorded from one electrode, while the other was used for pacing. In the first 10 patients examined, however, only one electrode was used, and the P waves had to be detected from the extremity leads I, II, III and the precordial lead V.

Medtronic external pacemaker type 5837 was used. The electrode position was accepted when pacing threshold was 3 mA or less. The atria were paced with an impulse twice threshold value at rates of 100, 120, and 140/min. At each rate, pacing was discontinued after 60 sec, spontaneous rhythm recorded for 20 sec and pacing resumed for another 60 sec. These 2 observations were made for each pacing rate and the longest SRT observed after each pacing rate was used for further analyses. The interval between the fifth and sixth normal P wave after trial pacing was found to be representative for the basic atrial rhythm in most of the patients and was used in calculations as the patients' basal P-P interval. When atrial rhythm was irregular the average of the five following P-P intervals was used. P-P intervals of 5000 msec or more were defined as sinus arrest. ECG was recorded on Mangograph, paper speed 50 mm/sec, and the intervals were measured on the ECG paper. For the statistical analysis the paired *t*-test was used.

MATERIAL

SRT was recorded in 66 patients, 28 of whom had routine right heart catheterization in the preoperative investigation for coronary heart disease (CHD). Eleven patients with advanced AV block were examined in connection with registration of His bundle potentials or introduction of temporary pacemaker electrode. Seventeen patients

Table 1 Sinus node recovery time in patients with coronary heart disease

Pat. no.	SRT (msec)			SRT (% of basal P-P interval)		
	Atrial pacing rate			Atrial pacing rate		
	100	120	140	100	120	140
1	1130	1180	1150	138	128	121
	1140	1190	1130	119	118	117
3	1020	1220	1150	136	153	129
4	1000	1000	980	135	137	120
5	1160	1160	1190	134	129	~
6	920	940	900	11	170	112
7	1170	1030	1010	124	119	119
8	1000	950	950	133	177	178
9	1140	1040		128	133	~
10	1260	1230	1120	148	149	127
11	1100	1070	980	130	124	114
1	1120	1160	1200	135	126	130
13	980	920	1040	177	125	137
14	1030	1080		143	137	~
15	1280	1230	1360	145	158	134
16	990	930	950	137	137	138
17	860	890	1030	125	127	147
18	800	800	670	116	133	114
19	690	780		111	131	
20	~	830	820		143	138
1	770	980	830	114	126	134
III	1390	1380	1280	136	134	170
3	1340	1200	1220	137	176	164
4	1470	1440		128	130	
5	1330	1070	1330	123	91	139
26	1160	1150	1700	145	137	140
77	1190	1120	1020	147	143	142
28	1080	1170	1150	159	142	128
Mean	1090±36	1070±30	1070±34	131±2	131±	128±2

were examined due to episodes of paroxysmal tachyarrhythmia and 10 for suspected sinus node dysfunction.

The effect of β -receptor blockade and of verapamil on SRT was examined in 1 and 7 patients respectively with ID and presumably normal sinus node function.

RESULTS

Patients without symptoms of sinus node dysfunction

In the 28 patients with CHD and presumably normal sinus node function average SRT was 1080 msec. After an atrial pacing rate of 100/min mean SRT was 1090 msec, after a pacing rate of 140/min mean SRT was 1070 msec. These differences were, however, not significant (Table 1). Two patients (nos. 10 and 18) who developed anginal pain during atrial pacing at a rate of 140/min had shorter SRT at that rate. A positive correlation was found between SRT and the basal endogenous P-P interval (after a pacing rate of 100/min $R=0.88$ after a pacing rate of

170/min, $R=0.79$ after a pacing rate of 140/min, $R=0.67$). The mean SRT was about 130% of the basal P-P interval at all pacing rates.

Patients with A1 block

Eleven patients with advanced AV block were examined. One of them had a congenital and 10 an acquired AV block (Table II). The mean SRT was 1070 msec after a pacing rate of 100/min and 1190 msec after a pacing rate of 140/min. These differences were not statistically significant. Mean SRT for all observations was 1080 msec. In these patients the basal P-P intervals were often difficult to assess due to irregular atrial rhythm. In 4 patients P-P intervals were shorter if a QRS complex appeared inbetween the two P waves. In one case P waves tended to follow immediately after the start of QRS complexes, probably due to supraventricular extrasystoles and not to retrograde A1 conduction. In 6 patients the longest P-P interval did not follow immediately after termination of the atrial

Table II. Sinus node recovery time in patients with advanced AV block

Pat. no.	SRT (msec)			SRT (% of basal P-P interval)		
	Atrial pacing rate			Atrial pacing rate		
	100	120	140	100	120	140
1	1 140	1 170	1 240	146	131	141
2*	940	1 050	1 100	139	146	153
3	1 340	1 340	980	146	111	97
4*	1 40	1 200	1 320	1 4	108	123
5	1 300	1 200	1 140	129	124	114
6*	1 060	1 040	1 140	117	118	127
7	1 180	1 140	1 380	120	114	138
8	1 200	1 030	1 180	126	118	113
9	1 200	930	840	160	122	100
10*	1 070	1 410	1 460	135	178	185
11	1 160	1 320	1 360	129	145	166
Mean	1 170±30	1 170±90	1 190±60	134±4	129±6	132±8

* Shorter P-P intervals with time relation to ventricular systole. Unpredictable variations in P-P interval longest spontaneous P-P interval 1 700 msec. Congenital AV block. Supraventricular extrasystoles.

pacing in one or more registrations. One of these patients (no 3) had on one occasion a spontaneous P-P interval of 1 700 msec. In the other patients all spontaneous P-P intervals were less than 1 500 msec. In the patients with AV block SRT was on an average 131% of the basal P-P interval.

Patients with sick sinus syndrome

Ten patients with a clinical diagnosis of sick sinus syndrome were examined. The diagnosis was made before the SRT examination and was based on history and ECG recordings. Duration of symptoms and relevant clinical data are listed in Table III. The group included patients with marked tendency to sinus arrest as well as patients with moderate symp-

toms due to intermittent sinoatrial block. Eight of the patients had symptoms which warranted pacemaker implantation.

In this group SRT varied greatly between patients and in many of them between registrations (Table IV). In 10 of the 10 patients SRT was shorter than 1 900 msec in all registrations. In one of them however P-P interval of more than 1 900 msec duration were observed during the next 8 sec after discontinuation of atrial pacing. In 6 cases long P-P intervals, even sinus arrest were observed a few seconds after atrial pacing as shown in Fig. 1. When episodes of sinus arrest (SRT>5 sec) were excluded a mean SRT of 1 780 msec was observed. Sinus arrest was seen in 3 patients after pacing at a

Table III. Clinical data on patients with sick sinus syndrome

Pat. no.	Age (y)	Symptoms	Duration (y)	Tachyarrhythmias	AV block	Permanent pacemaker
1	70	Congestive heart failure	3	Parox. flutter	2:1	Yes
2	64	Dizziness	3			Yes
3	53	Dizziness	3	Parox. flutter		Yes
4	66	Dizziness	20	Parox. nodal tach.		No
5	76	CHD	1	Parox. atrial tach.		Yes
6	80	Dizziness	9			Yes
7	77	Dizziness	1		Atrial pacing 120 Wenckebach	No
8	64	Dizziness, syncope	14	Parox. flutter	Atrial pacing 100 Wenckebach	Yes
9	53	Syncope	6	Parox. flutter		Yes
10	64	Dizziness	6			Yes

Table IV Sinus node recovery time (msec) in 10 patients with sick sinus node

Pat. no.	Atrial pacing rate		
	100	120	140
1	1 420 4 600	-	Sinus arrest 1 350
2	Sinus arrest	-	Sinus arrest Sinus arrest
3	-	Sinus arrest Sinus arrest	1 650 -
4	1 550 1 700	1 320 1 430	3 800 4 400
5	800 2 150	4 500 1 000	940 1 900
6	1 360 1 360	1 320 1 200	1 360 1 200
7	1 220 1 600	1 050 1 580	1 100 1 500
8	3 260 2 730	Sinus arrest Sinus arrest	Sinus arrest Sinus arrest
9	1 170 1 370	1 015 1 120	1 150 1 070
10	Sinus arrest Sinus arrest	Sinus arrest Sinus arrest	Sinus arrest Sinus arrest
Mean	1 870+/-270	1 350+/-330	1 920+/-30

In one or more of the registrations one of the following P-P intervals was longer than the SRT. Episodes of sinus arrest (SRT>5000 msec) have been excluded.

rate of 100/min and in 4 patients after pacing at a rate of 140/min

Patients with paroxysmal tachyarrhythmias

Seventeen patients with paroxysmal tachyarrhythmias were examined (Table V). Fifteen had SRT

shorter than 1500 msec at all pacing rates. Two of the patients (nos 4 and 8) both with CHD had SRT of 1500 msec or more. Patient 8 had left ventricular aneurysm and paroxysmal ventricular tachycardia. During 2 weeks observation in a CCU sinus arrest, SA block or extreme bradycardia had never been observed. In patient 4 who had had paroxysmal supraventricular tachycardia for many years one possible episode of sinus arrest had been seen on the oscilloscope screen during observation in a CCU.

Irregular atrial rhythm after termination of atrial pacing was very common in these patients usually due to supraventricular extrasystoles in some cases to ventricular extrasystoles and retrograde P waves. In one patient atrial pacing was followed by a supraventricular tachycardia for a few seconds. In 6 patients the longest P-P interval did not follow immediately after termination of the atrial pacing in one or more of the registrations.

Effect of drugs on SRT

In 5 CHD patients SRT was recorded 10 min after β -receptor blockade (propranolol 5 mg i.v.). SRT increased from 1080+/-40 to 1300+/-50 msec and this increase was statistically significant ($p=0.001$). This prolongation of SRT corresponded to the bradycardia induced by the β -receptor blockade. The basal P-P intervals increased from 830 to 950 msec. The SRT as a percentage of the basal P-P intervals was unchanged (131% before, 133% after propranolol).

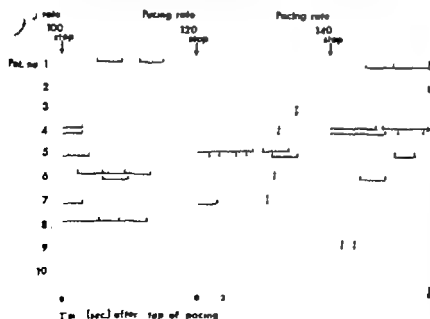


Fig. 1 Rhythm diagram in patients with sick sinus node. Atrial rhythm during the first 8 sec after atrial pacing (pacing rates 100, 120, 140/min) (—P waves, —P-P intervals longer than 1400 msec).

Table V Sinus node recovery time in patients with tachyarrhythmias

PAT=paroxysmal tachycardia, PAF=paroxysmal atrial fibrillation, VES=ventricular extrasystole, PVT=paroxysmal ventricular tachycardia, AES=atrial extrasystole

Pat. no.	Age (y.)	Type of tachyarrhythmia	SRT (msec)			SRT (% of basal P-P interval)		
			Atrial pacing rate			Atrial pacing rate		
			100	120	140	100	120	140
1	50	PAT	1 050	980	970	128	120	121
2	45	PAT	1 040	1 300	1 340	102	146	146
3	45	PAF	1 220	1 040	—	160	135	—
4	73	PAT	1 500	1 500	1 300	142	130	140
5	59	PAT	1 000	940	1 070	119	122	119
6	55	PAT	1 000	930	880	113	102	105
7	31	VES	1 160	1 190	1 160	135	138	132
8	62	PVT	1 530	1 820	—	115	127	—
9	61	VES	1 050	1 060	980	135	137	126
10	62		1 260	1 330	1 190	136	132	125
11	54	AES						
		Prov. AV block I	1 020	1 060	1 000	120	127	111
12	67	PVT	1 300	1 250	1 130	117	120	140
13	66	PAT	1 460	1 200	1 120	130	124	108
14	55	PAF	1 300	1 320	1 380	137	143	141
15	67	PAT	1 460	1 260	1 250	127	107	119
16	69	PAT	970	1 080	940	137	154	138
17	20	PAT	800	900	900	129	120	155
Mean			1 190+/-30	1 190+/-60	1 110+/-40	129+/-3.0	128+/-3.0	129+/-4.0

In 7 CHD patients SRT was recorded 10 min after verapamil (Isoplin® 5 mg i.v.). No significant effect was observed on the SRT. The basal heart rate was unchanged. SRT was 1 150 msec before and 1 180 msec after verapamil. The difference was not statistically significant. SRT as a percentage of the basal P-P interval was 134 before as well as after verapamil.

DISCUSSION

Recordings of SRT are easy to perform and can be done in connection with other invasive diagnostic procedures. It is advantageous to have an intracardiac ECG for recording of P waves because P waves in standard ECG leads sometimes are obscured by QRS or T waves. Atrial extrasystoles are also usually easier to detect from an intracardiac recording. In many patients a different duration of SRT can be observed from one registration to another and therefore more than one recording should be taken in each case.

In patients with no clinical dysfunction of the sinus node we found an average SRT of 1 080 msec and in no case an SRT longer than 1 500 msec. These figures may be taken as normal values for

SRT. On an average SRT was 130% of the basal P-P interval and in no case more than 160%. Our observations correspond fairly well with previously published results (1, 3, 5, 6). In some of the patients with clinically proved poor sinus node function we have recorded a normal SRT on one or more occasions. The recording of a normal SRT therefore does not exclude the possibility of a poorly functioning sinus node. In some instances the following spontaneous P-P intervals are longer than the SRT and may exceed 1 500 msec. We have observed that even sinus arrest may occur a few seconds after atrial pacing.

It was previously found by others (3, 4) that the duration of SRT normally is not related to the atrial pacing rate. Mandel et al. (3) however observed a reduction in SRT after atrial pacing rates of more than 140/min. We made the same observation of shortened SRT in patients who developed angina pectoris at atrial pacing rates of 140/min. Narula et al. (4) observed that SRT lengthened as the pacing rate increased in patients with sinus bradycardia. In the present study sinus arrest occurred more frequently after rapid atrial pacing than after atrial pacing at a moderate rate in patient with sick sinus syndrome.

To our knowledge the effect of β -receptor blockade on SRT in man has not been published before. In the present study a significantly prolonged SRT was observed and this prolongation was probably related to the bradycardia induced by the β -receptor blockade. Verapamil is reported to prolong SRT (2). In this study however verapamil had no effect either on SRT or on the spontaneous atrial rate.

REFERENCES

- 1 Dalinga, R. C., Rosen, K. M. & Rahimtoola, S. H., Normal conduction intervals and responses in sixty-one patients using His bundle recording and atrial pacing. *Chest* 64: 55, 1973.

- 2 Hummel M. H., Krasnicka J., Rydén, L. & Holmberg, S., Action of verapamil on sinus node, atrioventricular and intraventricular conduction. *Brit. Heart J.* 35: 734, 1973.
- 3 Mardel, W., Hayakawa, H., Denzig, R. & Marcus, H. S., Evaluation of sino-atrial node function in man by overdrive suppression. *Circulation* 44: 39, 1971.
- 4 Narula, O. S., Samet, P. & Javier, R. P., Significance of the sinus-node recovery time. *Circulation* 45: 140, 1972.
- 5 Rich, J. M., Melsner, M. H., Fontana, M. E. & Woolley, C. F., Electrophysiologic stress test in man: Sino-atrial node suppression and recovery. *J. Lab. clin. Med.* 78: 805, 1971.
- 6 Rosen, K. M., Loeb, H. S., Sumo, M. Z., Rahimtoola, S. H. & Gunnar, R. M., Cardiac conduction in patients with symptomatic sinus node disease. *Circulation* 43: 836, 1971.

MINOXIDIL—HAEMODYNAMIC AND CLINICAL EXPERIENCES WITH A NEW PERIPHERAL VASODILATOR

Rune Sannerstedt, Leif Brorson Göran Berghand and Lars Werkö

*From the Department of Medicine I Sahlgrenska University Hospital,
Göteborg Sweden*

Abstract Minoxidil, a new peripheral vasodilator given orally to hypertensive men in single doses of 5–25 mg, produced no haemodynamic changes within one hour after administration. After repeated oral doses within 24 hours to a total of 15–45 mg and after 10 mg t.i.d. orally for one week, moderate decreases in BP were seen concomitant with tendencies to increased heart rate and cardiac output. Clinically oral minoxidil 10–30 mg daily in combination with diuretics and adrenergic β -receptor blocking agents achieved an improved BP control in five patients with sustained arterial hypertension and unsatisfactory response to previous treatment. However in four of the five patients minoxidil had to be withdrawn because of side-effects. It is concluded that minoxidil, producing hypotensive effect of slow onset may find place as a therapeutic addition to symptomatic patients with severe and therapy-resistant hypertensive cardiovascular disease provided adequate measures are taken to counteract side-effects especially water retention and development of oedema.

The theoretically attractive approach of combining adrenergic β -receptor blocking agents with peripheral vasodilators for treatment of arterial hypertension has aroused new interest in the development of compounds with peripheral vasodilating properties (2 3 6 7 13 14 15).

One such compound is minoxidil a piperidino-pyrimidine derivative, shown in animal experiments to possess a direct relaxant effect on vascular smooth muscle (1). In man minoxidil given orally in doses of 15–80 mg a day for one week on ses haemodynamic changes characteristic of peripheral vasodilators, i.e. decreased BP concomitant with increases in heart rate and cardiac output (3). Preliminary clinical studies with minoxidil have yielded promising results in hypertensive patients usually in combination with adrenergic β -receptor blocking agents and diuretics (4 8 10).

The present investigation was undertaken to evaluate minoxidil further. Five hypertensive men were studied haemodynamically before and after single and repeated doses of the drug. In a pilot study the clinical effects were observed in another five patients in whom previous therapy had been unsatisfactory.

MATERIAL AND METHODS

Haemodynamic study

Eight male in-patients between the ages of 30 and 55 years with benign essential hypertension gave their informed consent to participate in the study. One of these (no 11) had cardiac enlargement on X-ray (600 ml/m² BSA) with slight signs of pulmonary congestion, otherwise no patient had signs of cardiac or renal insufficiency. Four patients were previously treated. Propranolol had been given for short periods to three patients, but was withdrawn one week or more before the study. The remaining patient had received total of 80 mg propranolol the day before the first study.

In six patients the acute effects of single dose of minoxidil were studied, the systemic haemodynamics at rest in recumbency being determined before and one hour after an oral dose. Four patients in groups of two were given 5 and 10 mg, respectively and two patients received 15 and 25 mg, respectively of minoxidil administered in capsules containing 2.5 mg.

Without any other treatment in the morning, five of these patients had their systemic haemodynamics in recumbency restudied one week later (in one case seven weeks later) after three oral doses of minoxidil within the preceding 24 hours, the last dose being given in the morning of the day of the study. The same four patients as above in groups of two got total of 15 and 30 mg, respectively. The patient who had previously received 15 mg in single dose was given total of 45 mg.

Two patients were studied at rest and during standardized bicycle ergometer exercise (750 kpm/min) in the sitting position before and after oral minoxidil 10 mg t.i.d. for one week.

Table 1 Clinical effects of minoxidil

Auscultatory BP in the lying and standing (italics) positions, and HR in recumbency. Averages of last three recordings before entering the study and of three measurements after 1, 2 and 4 weeks on minoxidil

Alpr=alprenolol Chlor=chlorthalidone Clon=clonidine Furo=furosemide Guan=guanethidine HyChl=hydrochlorothiazide Hydr=hydralazine, Minox=minoxidil, Propr=propranolol

Before minoxidil				During minoxidil				Duration of treatment (weeks)	Comments
Pat. no.	Age (y)	BP (mmHg)	HR (beats/min)	Treatment (mg/d.)	BP (mmHg)	HR (beats/min)	Treatment (mg/d.)		
1	53	182/127 <i>180/127</i>	63	Propr 480 Hydr 150 Chlor 50	196/120 <i>176/116</i>	116	Minox 40 Chlor 50-100 Alpr 1800	5	Stopped minoxidil because of dyspnoea and dependent oedemas
2	46	232/124 <i>193/126</i>	56	Alpr 1200 Hydr 200 Chlor 50 Clon 0.45	180/111 <i>176/113</i>	69	Minox 20 Chlor 50 Alpr 800 Clon 0.15 Furo 80	6	Stopped minoxidil because of dependent oedemas
3	57	206/118 <i>211/121</i>	82	Propr 480 Chlor 50 Guan 100	163/105* <i>177/107*</i>	84*	Minox 10 Chlor 50 Alpr 800	1	Stopped minoxidil because of dyspnoea and angina pectoris
4	60	223/114 <i>218/115</i>	56	Propr 480 Hydr 150 HyChl 50	208/100 <i>158/102</i>	71	Minox 50 Chlor 50 Alpr 1200	8	Stopped minoxidil because of aggravated claudication
5	54	206/130 <i>204/137</i>	84	Propr 370 Chlor 50	160/102 <i>174/120</i>	87	Minox 10 Chlor 50 Alpr 800	18	No side-effects
Mean		210/123 <i>201/123</i>	68		182/108 <i>172/112</i>	85		8	

*Averages of two measurements after 1 and 2 weeks on minoxidil.

no examination procedure for obtaining haemodynamic data and the techniques used for their analyses have been described in detail elsewhere (12). Briefly the patients were brought to the laboratory in the morning after overnight fast. Under local anaesthesia polyethylene catheters were inserted into the brachial artery and an antecubital vein. The venous catheter was advanced under fluoroscopy to the superior vena cava for subsequent central injections of dye. The intra-arterial BP was registered on an Ultraleite recorder by means of strain gauges (Statham P23Db) and the mean arterial BP was determined by electrical integration. The cardiac output (\dot{Q}) was determined using dye-dilution technique with brocresulphalein as the indicator and with intermittent sampling of arterial blood. An ECG was continuously monitored, it was registered on the Ultraleite recorder during the pressure recordings and during the \dot{Q} procedure and used for calculations of the heart rate (HR). From the data for HR, mean brachial artery BP and \dot{Q} the stroke volume (SV) and systemic vascular resistance (SVR) were calculated and expressed in ml/beat, and arbitrary units, respectively.

After insertion of the catheters the patients rested for

30 min before the first measurements at rest were made with the patients in recumbency on the fluoroscopy table or comfortably sitting in an arm-chair. \dot{Q} during exercise was determined during the final minute of the exercise period, which lasted 10 min.

Clinical study

Five male out-patients 46-60 years of age, were given minoxidil because of unsatisfactory response to other forms of antihypertensive treatment. Four of them had benign essential hypertension, one had benign sustained hypertension after reconstructive surgery for renal artery stenosis.

After withdrawal of previous treatment, minoxidil 5 mg orally b.i.d. was given together with alprenolol (Aptis[®]) and chlorthalidone (Hygroton[®]). The doses of minoxidil, alprenolol and chlorthalidone were then adjusted according to BP response and occurrence of side-effects especially tachycardia and dependent oedema, the highest dose of minoxidil given being 50 mg daily (Table 1). The observation period varied from 3 to 18 weeks (average 8), during which the auscultatory BP was measured at regular intervals with mercury manometer. Recordings were

Table II Haemodynamic effects of minoxidil

Individual findings in six patients at rest in recumbency before, one hour after a single oral dose and after repeated oral doses within 24 hours

Pat. no.	Phase	HR (beats/min)	Brachial artery BP (mmHg)			\dot{Q} (l/min)	SV (ml/beat)	SVR (U)
			Systolic	Mean	Diastolic			
6	Before	72	186	125	84	8.7	121	14.4
	5 mg p.o.	68	180	124	89	7.9	116	15.7
	15 mg p.o./24 h	72	158	109	80	7.4	103	14.7
7	Before	54	162	116	91	5.0	93	23.3
	5 mg p.o.	60	152	112	88	5.5	92	20.5
	15 mg p.o./24 h	66	131	105	81	6.3	96	16.7
8	Before	60	176	123	98	5.6	80	22.4
	10 mg p.o.	62	173	123	96	5.6	90	22.0
	30 mg p.o./24 h	72	161	122	94	7.6	106	16.0
9	Before	72	212	149	111	6.9	96	21.6
	10 mg p.o.	70	202	143	110	6.7	96	21.4
	30 mg p.o./24 h	76	205	144	111	7.8	103	18.5
III	Before	48	197	128	94	6.5	135	19.7
	15 mg p.o.	48	181	124	91	7.5	156	16.6
	45 mg p.o./24 h	54	166	116	85	7.2	133	16.2
II	Before	70	237	168	130	6.7	96	25.1
	225 mg p.o.	70	231	164	122	7.2	103	22.8

obtained after 5 min of rest in recumbency and III the erect position after 1-2 min

RESULTS

Haemodynamic studies

The haemodynamic findings are presented in Table II and Figs 1-3

No consistent changes indicative of drug effect, were seen one hour after administration of a single oral dose of 5-25 mg minoxidil (Fig. 1)

After repeated oral doses within 4 hours, all five patients had a lower brachial artery BP (-7 to -31 mmHg) concomitant with a tendency to increased HR and \dot{Q} and the calculated SVR was lower in four of the patients (Fig. 2) There was however no obvious dose-response relationship

The same tendencies were also seen in the two patients restudied after oral treatment with minoxidil for one week but the changes observed were slight throughout (Fig. 3)

Clinical study

All five patients showed a decrease in BP as judged from the average of recordings after 1, 2 and 4 weeks (Table I) but none became normotensive. The average BP reduction in the recumbent position was 28/15 and in the erect position 29/13 mmHg.

The pulse rate at rest rose in every patient from an initial average of 68 to 85 beats/min.

The average weight increased from 85.5 kg during the previous treatment to an average of 89.7 kg after 1, 2 and 4 weeks on minoxidil. In four patients the treatment had to be discontinued because of side effects in the form of oedemas, increased symptoms of angina pectoris and claudication. Thus, in one patient (no. 2) chlorthalidone 50 mg daily did not prevent the development of dependent oedemas and furosemide had to be added. In another patient (no. 1) sustained tachycardia was present in spite of continuous administration of alprenolol in adequate doses. No abnormalities in routine blood liver and kidney tests were noted

DISCUSSION

Pharmacokinetic studies in hypertensive patients have shown minoxidil to be rapidly and completely absorbed after oral administration with maximal plasma concentrations occurring at one hour (5). Based on this, haemodynamically demonstrable effect was expected to appear in the present study one hour after a single oral dose. However no immediate cardiovascular effects were noticed even when the single dose of minoxidil had been increased up to 25 mg, and only inconsistent

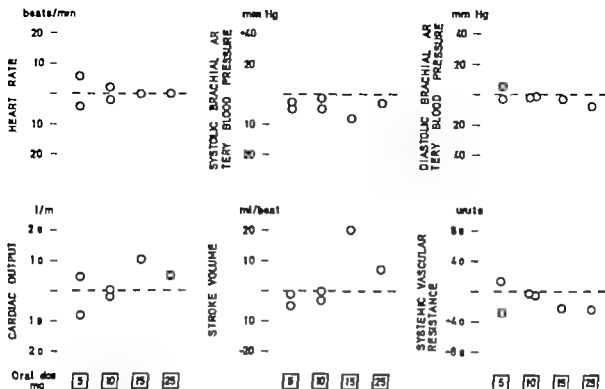


Fig 1 Haemodynamic responses to single doses of minoxidil 5–25 mg orally. Hypertensive patients studied at rest in recumbency.

non-decisive changes were observed after repeated doses within 24 hours or after treatment for one

indicates a slow onset of action of minoxidil as to animal findings where a dose maximum hypotensive effect occurred after 4–6 hours and lasted for 24–48 hours after single oral doses (1). It is also contrary to the pharmacodynamic properties of other peripheral vasodilators such as hydralazines where the cardiovascular effects present themselves as BP reduction with a compensatory increase in \dot{Q} within 30 min after an oral dose of 100–150 mg (9).

A slow onset of action was also obvious in the small group of hypertensive patients followed clinically. Animal studies have shown a specific retention of minoxidil in the femoral artery and aorta to occur after intraperitoneal application (11) and theoretically the slow onset of action could be due to a continued accumulation of minoxidil in the peripheral tissues particularly the vessel walls.

In agreement with the results of others (4, 8, 10) the present pilot study confirmed minoxidil to be

of therapeutic value in conjunction with other anti-hypertensive agents like diuretics and adrenergic β -receptor blocking agents for treatment of therapeutically difficult cases.

The improved BP control was achieved at the expense of side-effects requiring withdrawal of minoxidil in four of five patients and this discouraged us from extending the present type of trial to cover a larger number of patients. Concomitant therapy with saluretics and adrenergic β -receptor blocking agents was not enough to prevent development of oedemas in two patients and precipitation of angina pectoris in one patient. One of the oedematous patients also reacted with persistent tachycardia in spite of a high dose of alprenolol. Sodium retention has been demonstrated to be a consistent feature in patients treated with minoxidil and precipitation of myocardial ischaemia has also been described (3, 10).

Side-effects of other kinds e.g. hypertrichosis have also been reported (4, 10) and in addition minoxidil has in dogs been shown to produce necrosis in the right atrium (1). Therefore it seems

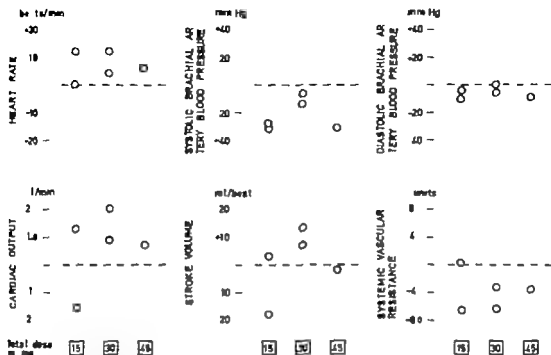


Fig. 2 Haemodynamic changes in hypertensive patients given total of 15-45 mg minoxidil within 24 hours before the study

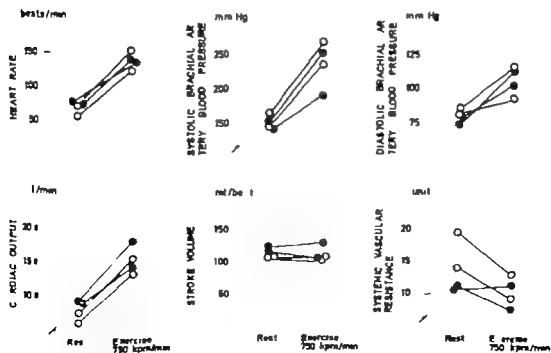


Fig. 3 Systemic haemodynamics at rest and during exercise treatment with minoxidil 10 mg t.i.d. for one week in two hypertensive patients.

unlikely that minoxidil will become a drug suitable for routine treatment of initially symptom-free patients with benign arterial hypertension.

Minoxidil may however, as pointed out by several authors (3-8, 10) be justified as a therapeutic addition to patients with very severe and drug-resistant arterial hypertensive disease. Adequate measures must then be taken to counteract side effects, especially water retention and development of oedemas.

REFERENCES

1. DuCharme D. W., Freyburger W. A., Graham, B. E. & Carlson, R. H. Pharmacologic properties of minoxidil. A new hypotensive agent. *J. Pharmacol. exp. Ther.* 184: 662, 1973.
2. Gilmore, E. W. J. & Chidsey C. A. Treatment of essential hypertension with a new vasodilator in combination with beta-adrenergic blockade. *New Engl. J. Med.* 282: 521, 1970.
3. Gottlieb T. B., Katz, F. H. & Chidsey C. A., III. Combined therapy with vasodilator drugs and beta-adrenergic blockade in hypertension. A comparative study of minoxidil and hydralazine. *Circulation* 45: 571, 1972.
4. Gottlieb, T. B., Thomas, R. C. & Chidsey C. A., III. Pharmacokinetic studies of minoxidil. *Clin. Pharmacol. Ther.* 13: 436, 1972.
5. Kafka, M. & Frick, M. H. Combined dihydralazine and propranolol in the treatment of hypertension. *Int. J. clin. Pharmacol.* 4: 111, 1970.
6. Koch-Weser J. Vasodilation for vasospastic hypertension. *New Engl. J. Med.* 289: 213, 1973.
7. Leading article: New vasodilator drugs for hypertension. *Brit. med. J.* 4: 185, 1973.
8. Lamas, C. J. & Freis, E. D., Minoxidil in severe hypertension with renal failure. Effects of its addition to conventional antihypertensive drugs. *Amer. J. Cardiol.* 31: 355, 1973.
9. Moyer J. H. Hydralazine (Apresoline®) hydrochloride. Pharmacological observations and clinical results in the therapy of hypertension. *Arch. Int. Med.* 91: 419, 1953.
10. Pettinger W. A. & Mitchell, H. C. Minoxidil—an alternative to nephrectomy for refractory hypertension. *New Engl. J. Med.* 289: 167, 1973.
11. Pluss, R. G., Orcutt, J. & Chidsey C. A. Tissue distribution and hypotensive effects of minoxidil in normotensive rats. *J. Lab. clin. Med.* 79: 679, 1972.
12. Sannerstedt, R., Hemodynamic response to exercise in patients with arterial hypertension. *Acta med. scand. Suppl.* 458, 1966.
13. Sannerstedt, R., Stenberg, J., Johansson, G. & Werkö, L., Hemodynamic interference of alprenolol with dihydralazine in normal and hypertensive man. *Amer. J. Cardiol.* 28: 316, 1971.
14. Sannerstedt, R., Stenberg, J., Vedin, A., Wilhelmsson, C. & Werkö L., Chronic beta adrenergic blockade in arterial hypertension. *Amer. J. Cardiol.* 29: 718, 1972.
15. Werkö, L., New treatment for hypertension. *Europ. J. clin. Pharmacol.* 3: 61, 1971.

THE EFFECT OF HYDROCHLOROTHIAZIDE AND AMILORIDE ADMINISTERED TOGETHER ON MUSCLE ELECTROLYTES IN NORMAL SUBJECTS

Jonas Bergström with the technical assistance of Anne-Marie Fridén

From the Department of Nephrology and the Rheumatological and Metabolic Research
Laboratory St Erik's Hospital, Stockholm, Sweden

Abstract Hydrochlorothiazide 150 mg daily and amiloride, 15 mg daily have been administered together to 10 normal subjects during one week. Muscle biopsies were performed before and after the period of administration of the diuretics and the material was analysed for water and electrolytes. The body weight decreased by 1.9 kg. The serum sodium, chloride and potassium concentrations decreased significantly and standard bicarbonate and blood pH increased. In muscle tissue extracellular water chloride and sodium contents and intracellular sodium concentration decreased but the muscle potassium content, the intracellular potassium concentration and the muscle magnesium content were unchanged. A comparison with the results of an earlier study in which hydrochlorothiazide was given without amiloride showed that the intracellular potassium depletion and the increase in intracellular sodium concentration, observed with the benzothiadiazine diuretic could be fully prevented by simultaneous administration of amiloride. The degree of hypokalaemia and alkalosis was also smaller with hydrochlorothiazide+amiloride than with hydrochlorothiazide alone.

Benzothiadiazine diuretics increase the excretion of potassium and thus may induce hypokalaemia and intracellular loss of potassium. This has been attributed to inhibition of sodium reabsorption in the distal tubules proximal to the ion exchange site permitting a greater supply of sodium for utilization of the potassium-sodium exchange mechanism (2).

The diuretic agent amiloride acts at the distal renal tubules where it interferes with the exchange of sodium for potassium (1). When given together with a benzothiadiazine diuretic, it further enhances the natriuretic action and prevents the potassium loss (15).

Bergström and Hultman (5) using a needle biopsy technique could demonstrate that administration of hydrochlorothiazide (150 mg daily) to normal subjects during one week resulted in a significant decrease in muscle potassium. The present study was

undertaken in order to find out whether amiloride administered together with hydrochlorothiazide could prevent the expected intracellular potassium loss induced by the thiazide.

MATERIAL AND METHODS

Ten healthy volunteers, aged 24-35, were examined by means of needle biopsies taken from an quadriceps femoris before and after 7 days administration of hydrochlorothiazide (150 mg, 3x50 mg, daily) and amiloride (15 mg, 3x5 mg, daily). Venous blood was collected from cubital vein at the time of biopsy care being taken to avoid errors due to stumping and handling of the samples (10).

Arterialized capillary blood from finger tip was collected for determination of pH, P_{CO_2} and standard bicarbonate. The body weight was recorded before and after the administration period of the diuretics. The subjects who carried on their usual daily work, were not on a controlled diet, and the water and salt intake was not restricted.

The muscle biopsies were performed between 8 and 10 a.m. after an overnight fast.

The needle biopsy specimens were taken alternating from both legs before and after the period of diuretic administration. On each occasion sampling was done at two different sites: 1) 14-16 cm proximally to patella and 2) 20-22 cm proximally to patella. Each specimen which weighed 20-80 mg, was divided into 2-4 pieces, weighing 10-20 mg. Visible fat and connective tissue were rapidly removed by dissection and the specimens were weighed on an electromagnetic balance. The specimens were dried at 90°C and weighed. Neutral fat was extracted with petroleum ether and the pieces were weighed again. The water and fat contents were calculated. The details of the biopsy technique, weighing procedure and fat extraction have been described earlier (3). Sodium, potassium and magnesium contents were determined by atomic absorption spectrophotometry and chloride was measured by electrometric titration (6). Serum electrolytes and protein were analysed according to routine method (Autochem 1) in the Clinical Laboratory. Separate samples of heparinized plasma were used for potassium and magnesium determination by emission and absorption flame spectrophotometry.

Table I The effect of hydrochlorothiazide (150 mg daily) and amiloride (15 mg daily) during 1 week on body weight and serum or plasma values in 10 normal subjects

B=before A=after

Subj no.	Age (y)	Sex	B. wt. (kg)	Protein (g/l)	Blood pH	Pco ₂ (mmHg)	Standard HCO ₃ ⁻ (mEq/l)	Cl ⁻ (mEq/l)	Na (mEq/l)	K (mEq/l)	Mg ⁺⁺ (mEq/l)
1 B	28	♂	64.2	7*	7.41	40.5	24.8	104	143	4.3	1.5
A			64.1	71	7.40	40.5	24.8	101	141	3.8	1.8
2 B	35	♂	87.0	80	7.41	38.5	24.5	103	143	4.1	-
A			83.7	87	7.48	37.0	27.0	95	134	3.5	1.9
3 B	25	♂	72.8	71	7.42	41.5	26.0	104	144	4.5	-
A			70.3	78	7.43	35.0	25.0	98	140	3.8	1.8
4 B	33	♀	62.2	78	7.40	37.0	23.0	106	144	4.0	1.5
A			60.3	81	7.46	34.0	25.0	98	141	3.7	1.4
5 B	24	♀	55.4	74	7.49	34.0	27.0	103	142	4.6	1.6
A			54.1	79	7.50	36.0	29.0	95	141	3.5	1.8
6 B	23	♂	74.9	78	7.40	35.0	23.0	104	143	4.0	1.8
A			72.8	80	7.45	38.0	27.0	97	139	3.5	2.1
7 B	25	♂	69.5	73	7.42	39.0	25.0	101	142	4.7	1.5
A			69.0	72	7.46	41.0	29.0	104	140	3.8	1.7
8 B	35	♂	78.5	69	7.43	41.0	27.0	106	141	3.9	1.6
A			75.0	78	7.45	37.0	27.0	98	137	3.8	1.5
9 B	31	♀	58.0	74	7.43	30.0	22.0	106	144	4.5	1.6
A			56.1	76	7.47	37.0	28.0	96	137	3.4	1.9
10 B	29	♀	47.3	72	7.45	35.0	25.0	108	144	3.7	1.5
A			45.3	73	7.46	36.0	26.0	97	137	3.6	1.4

Table II The effect of hydrochlorothiazide (150 mg daily) and amiloride (15 mg daily) during 1 week on muscle water and electrolytes in 10 normal subjects

B=before A=after tot=total, e=extracellular i=intracellular

	Per 100 g fat-free solids							Per l H ₂ O _i	
	H ₂ O _{tot} (ml)	H ₂ O _e (ml)	H ₂ O _i (ml)	Cl ⁻ (mEq)	Na (mEq)	K (mEq)	Mg ⁺⁺ (mEq)	Na _i (mEq)	K _i (mEq)
1 B	343	34.6	308	5.38	7.83	46.35	8.96	10.417	149.3
A	336	27.7	308	4.45	6.85	46.42	9.04	9.596	150.9
2 B	317	28.7	290	4.61	8.20	44.70	9.25	14.154	154.5
A	331	29.2	301	4.37	7.26	45.19	9.12	11.125	150.4
3 B	325	30.8	294	5.20	7.90	47.02	9.06	10.596	159.6
A	332	24.7	307	4.02	6.19	47.11	9.44	8.965	154.4
4 B	341	44.4	297	6.64	10.61	46.41	8.91	14.446	156.5
A	330	34.7	314	5.16	8.85	46.34	9.23	11.660	149.4
5 B	352	43.6	311	6.40	10.13	47.79	9.01	12.742	154.0
A	355	46.1	318	5.16	8.56	47.80	9.06	11.750	151.1
6 B	331	25.4	305	4.33	6.96	46.67	9.01	10.928	153.2
A	319	26.4	293	4.11	7.38	46.57	8.92	12.626	158.9
7 B	333	43.2	288	6.13	9.14	46.72	9.00	13.014	162.2
A	325	35.4	290	5.40	7.63	47.91	9.39	8.422	165.5
8 B	339	52.1	287	7.46	10.47	46.41	8.74	12.290	161.8
A	333	42.9	291	5.94	8.17	46.85	8.64	10.103	161.7
9 B	355	56.6	300	8.07	11.48	47.59	8.70	11.132	159.2
A	328	32.2	293	4.55	8.10	45.92	8.67	12.670	157.1
10 B	330	39.1	291	6.07	10.52	47.33	8.82	16.889	162.7
A	329	31.4	299	4.64	8.24	47.47	8.94	13.328	159.2

Table III Body weight serum or plasma values muscle water and electrolytes before and after hydrochlorothiazide (150 mg daily) and amiloride (15 mg daily) administration during 1 week in 10 normal subjects

Abbreviations as in Table II

	Mean		Differ- ence	t	Sig- nifi- cance
	B	A			
B wt. (kg)	67.0	65.1	-1.9	5.62	
<i>Serum or plasma values</i>					
Protein (g/l)	74	78	+4	3.09	
Blood pH	7.43	7.46	+0.03	4.17	**
PCO ₂ (mmHg)	37.2	37.2	0	-	-
Standard HCO ₃ ⁻ (mEq/l)	24.7	26.8	+2.1	2.99	
Cl ⁻ (mEq/l)	105	98	-7	5.20	
Na ⁺ (mEq/l)	143	139	-4	5.26	**
K ⁺ (mEq/l)	4.2	3.6	-0.6	3.09	***
Mg ²⁺ (mEq/l)	1.6	1.7	+0.1	1.87	-
<i>Muscle water and electrolytes</i>					
Per 100 g fat-free solids					
H ₂ O _{tot} (ml)	336	334	-2	0.74	-
H ₂ O _i (ml)	39.9	32.1	-7.8	3.53	**
H ₂ O _e (ml)	297	301	+4	1.52	-
Cl ⁻ (mEq)	6.0	4.8	-1.2	4.25	**
Na ⁺ (mEq)	9.3	7.7	-1.6	5.03	**
K ⁺ (mEq)	46.7	46.7	0	0.02	-
Mg ²⁺ (mEq)	9.0	9.1	+0.1	1.59	-
Per l H ₂ O _i					
Na ⁺ (mEq)	12.6	11.0	-1.6	2.48	
K ⁺ (mEq)	157	156	-1	1.14	

0.01 < p < 0.05 ** 0.001 < p < 0.01 p < 0.001

metry respectively. Standard bicarbonate, pH and PCO₂ were determined in whole blood by the Astrup micro-method (13). Tissue water and electrolyte contents were referred to 100 g fat-free solids.

The determination of extra- and intracellular water was based on the chloride method. Chloride is freely diffusible across the skeletal muscle fibre membrane and is distributed according to Nernst's equation (8). Taking the resting membrane potential of muscle in normal man to be 87.2 mV (7), the Cl_i/Cl_e ratio calculated from Nernst's equation will be 26/1. If the total water and chloride content of the muscle tissue and the extracellular chloride concentration (obtained by correcting the plasma chloride concentration for a Donnan factor and a factor for plasma water (3)) are known, extra- and intracellular water volumes and intracellular electrolyte concentrations can be calculated (4-9).

RESULTS

General condition

Three subjects complained of slight to moderate fatigue and one had leg cramps. Three subjects

experienced increased appetite and two increased thirst. No severe side-effects were observed.

Body weight serum electrolytes and protein and acid-base data (Tables I, II and III)

The body weight decreased. A slight fall in sodium concentration and a more pronounced fall in chloride concentration were observed. The potassium concentration decreased by 11.6 mEq/l which was highly significant. An increase in standard bicarbonate and blood pH also occurred.

Muscle water and electrolytes

Significant decreases in extracellular water chloride and sodium contents occurred. The muscle potassium content and the intracellular potassium concentration were not changed but the intracellular sodium concentration decreased slightly. The muscle magnesium content was unchanged.

Comparison between hydrochlorothiazide + amiloride and hydrochlorothiazide without amiloride (Table IV)

The present results were compared with the results of a similar study presented earlier in which ten normal subjects were given 150 mg hydrochlorothiazide daily during one week (5).

The mean decrease in body weight was exactly the same (1.1 kg) with both regimens indicating that the degree of dehydration induced was similar. The fall in extracellular potassium concentration and the degree of metabolic alkalosis (rise in standard bicarbonate) was greater with hydrochlorothiazide than with hydrochlorothiazide + amiloride. Hydrochlorothiazide administration resulted in a significant fall in muscle potassium and intracellular potassium concentration, and a significant increase in intracellular sodium concentration, whereas hydrochlorothiazide + amiloride had no effect on muscle potassium but induced a slight decrease in intracellular sodium concentration, i.e. an effect which was opposite to that of hydrochlorothiazide alone.

According to our records hydrochlorothiazide alone appeared to induce more pronounced side effects than hydrochlorothiazide + amiloride (see 'General condition'). Thus with hydrochlorothiazide no less than six subjects complained of severe fatigue, vertigo and nausea and two suffered from constipation. Increased thirst was experienced

Table IV Comparison between the effect of hydrochlorothiazide and hydrochlorothiazide + amiloride during one week in 10 normal subjects

	Plasma (mEq/l)			Muscle		
	ΔNa	ΔK	ΔHCO_3	Pat-free solids (mEq/100 g) ΔK	Intracellular water (mEq/l)	
					ΔNa^+	ΔK
A Hydrochlorothiazide (150 mg/d.)	-0.9	-1.11	+3.6	-2.7	+4.8	-12.5
B Hydrochlorothiazide (150 mg/d.) + amiloride (15 mg/d.)	-4.3	-0.60	+2.1	-0.06	-1.6	-1.4
A-B (1)	3.16	3.83	1.55	3.12	5.31	3.91
Significance	ns	**	-	ns		**

$$0.001 < p < 0.01 \quad ; \quad p < 0.001$$

by seven subjects. It is conceivable that mild potassium depletion was a factor of importance for the development of these side-effects.

DISCUSSION

In the present investigation the hydrochlorothiazide dose (150 mg daily) was larger than usually recommended for maintenance therapy of patients with cardiac insufficiency or hypertension. The proportion between the amiloride and hydrochlorothiazide doses was 1:10, i.e. the relation currently used in a fixed drug combination (Moduretic®) on the market. Our results demonstrate that by combining amiloride with hydrochlorothiazide it is possible completely to prevent the intracellular potassium loss which occurs in normal subjects after administration of thiazide alone.

However, amiloride could not fully prevent the development of hypokalemia and metabolic alkalosis induced by the thiazide diuretic, although the fall in plasma potassium concentration was less marked with hydrochlorothiazide + amiloride than with hydrochlorothiazide alone.

Another effect of a thiazide diuretic, namely an increase in the intracellular sodium concentration (5) could also be prevented by combining the thiazide with amiloride. Since the increase in intracellular sodium after thiazide administration probably occurs in exchange for potassium lost from the cells, our results further support the hypothesis that administration of amiloride preserves the intracellular electrolyte milieu during thiazide administration. The slight fall in extracellular potassium concentration is most probably a consequence of the co-existing slight metabolic alkalosis (12-14).

Intracellular potassium depletion, with consequent increase in intracellular sodium concentration, is known to increase the sensitivity to digitalis thus leading to increased risk of digitalis intoxication (11). Such intoxications are observed with increasing frequency due to the widespread use of diuretics which increase the excretion of potassium. Since amiloride is able to prevent the thiazide-induced fall in intracellular potassium concentration as well as the increase in intracellular sodium concentration, it appears to be a suitable drug for patients in whom the risk of digitalis intoxication must be considered. Also in other conditions where a patient is at risk of diuretic-induced potassium depletion, amiloride should be considered as an alternative to oral potassium supplementation.

Our results indicate that there is no risk of hyperkalemia when amiloride is combined with hydrochlorothiazide in the proportions used in this study, at least in subjects with normal renal function.

REFERENCES

1. Baer J. E. & Beyer K. H. Subcellular pharmacology of natriuretic and potassium-sparing drugs. In: *Drugs affecting kidney function and metabolism*. Progr. biochem. pharmacol. vol. 7, pp. 59-93. Karger, Basel, 1972.
2. Bank N. Physiological basis of diuretic action. *Ann. Rev. Med.* 19: 103, 1968.
3. Bergström J. Muscle electrolytes in man. Determined by neutron activation analysis on needle biopsy specimens. A study on normal subjects, kidney patients and patients with chronic diarrhoea. *Scand. J. clin. Lab. Invest. Suppl.* 68: 110, 1962.
4. Bergström J. & Bittar E. E. The basis of uremic toxicity. In: *The biological basis of medicine* (ed. E. E. Bittar & N. Bittar) vol. 6, pp. 495-544. Academic Press, New York, 1969.

5. Bergström J & Hultman, E.. The effect of thiazides, chlorthalidone and furosemide on electrolytes and muscle glycogen in normal subjects. *Acta med. scand.* 180: 363, 1966.
6. Bergström, J., Hultman E. & Solheim, S. II The effect of metfruside on plasma and muscle electrolytes and blood pressure in normal subjects and in patients with essential hypertension. *Acta med. scand.* 194: 427 1973
7. Bode, H. D. Riecker G. & Röhl, D. Messungen des Membranpotentials an einzelnen quergestreiften Muskelzellen des Menschen *in situ*. Normalwerte. *Klin. Wochr.* 41: 356, 1963.
8. Conway E. J.. Nature and significance of concentration solutions of potassium and sodium ions in skeletal muscle. *Physiol. Rev.* 37: 84 1957
9. Graham, J. A., Lamb, J. F. & Likton, A. L. Measurement of body water and intracellular electrolytes by means of muscle biopsy. *Lancet* 2: 1172, 1967
10. Hultman, E. & Bergström, J.. Plasma potassium de termination. *Scand. J. clin. Lab. Invest.* Suppl. 64: 87 1962.
11. Mason, H. T., Zelis, R., Lee, G., Hughes J. L., Spens, J. F. & Amsterdam, E. A.. Current concepts and treatment of digitalis toxicity. *Amer. J. Cardiol.* 27: 546 1971
12. Rooth, C. & Först, C.. The relation between hypokalaemia and alkalosis during administration of polythiazide and chlorthalidone. *Acta med. scand.* 176: 51 1964.
13. Siggaard Andersen, O. The acid-base status of the blood. *Scand. J. clin. Lab. Invest.* Suppl. 70, 1963
14. Simmons, D. H. & Avedon, M.. Acid-base alterations and plasma potassium concentration. *Amer. J. Physiol.* 197: 319 1959
15. Singh, B. N., Richmond, D. E., Wilson, J. D., Simmonds, H. A. & North J. D. K. Evaluation of MK 870: a new potassium-sparing diuretic. *Brit. med. J.* 1: 143 1967

TURNOVER OF PLASMA CHOLESTEROL IN PATIENTS WITH CHOLESTEROL GALLSTONES

L. Pedersen T. Aruffod and E. Heas Thaysen

*From the Departments of Medicine and Clinical Chemistry
Ålborg Hospital North, Ålborg Denmark*

Abstract Following the i. injection of about 30 μ Ci of 1- α -2- α - 3 H-cholesterol the specific activity decay of plasma cholesterol has been analysed in terms of a 2-pool model in eight patients with radiolucent gallstones and two healthy controls. In each subject some kinetic parameters were calculated, including the input into and the size of the rapidly exchangeable cholesterol pool. The results were compared with those in 16 controls from another study. Statistical analysis failed to demonstrate significant differences in any of the parameters between the gallstone patients and the combined controls, indicating that patients with cholesterol gallstones have normal input into and size of the rapidly exchangeable cholesterol mass. This conclusion is discussed in relation to similar studies, and it is speculated that gallstone patients may have decreased absorption of exogenous cholesterol.

In recent years the physicochemical basis of cholesterol gallstone formation has been studied intensively. It seems to be well established that the disease occurs in association with bile that contains an excess of cholesterol in relation to its content of bile acids and lecithin (1) and that the bile is lithogenic before it enters the gallbladder (16-19). Such an abnormality could arise from an increase in hepatic secretion of cholesterol from a decreased output of bile acids and/or lecithin or from a combination of these changes. So far most studies tend to show that a lithogenic bile is due preferably to a deficiency of bile acids relative to cholesterol (2-17, 18).

Lately however Grundy et al (7-8) have emphasized that concomitantly an increased cholesterol secretion may play an important role in the production of lithogenic bile. This observation was based upon direct measurements of duodenal biliary lipids in American Indians and the authors

think that their results are consistent with an increase in cholesterol synthesis.

In order to further explore this aspect we have estimated some kinetic parameters, including the size of the rapidly miscible pool and the input into this pool in a number of Caucasian patients with radiolucent gallstones.

MATERIAL AND METHODS

Eight patients with radiolucent gallstones in well functioning gallbladders, and two healthy control were studied (Table I). The examinations were performed on an outpatient basis in the course of about 10 weeks. No special diets were given and the weights of the patients remained unchanged. Any medical treatment for other diseases was maintained, but no drugs known to affect liver function or lipid metabolism were given. The liver function tests (serum aspartate aminotransferase, prothrombin time, alkaline phosphatase, serum protein electrophoresis and serum bilirubin) were normal in all patients and controls. None of the patients or controls had symptoms or signs of obstructive biliary disease.

Experimental procedure 1- α -2- α - 3 H-cholesterol was purchased from The Radiochemical Centre, Amersham, as benzene solution. This was dried under N and the tracer redissolved in ethanol. Purity exceeded 95% according to thin-layer chromatography. One ml of the ethanol solution was sterilized by passage through Milipore filter and added to 9 ml of Intralipid® (Vitrum) under sterile precautions. The specific activity was measured by liquid scintillation counting (Packard Tri Carb), and 1-3 ml injected to the patients and controls. The radioactivity dose was some 30 μ Ci. In three of the patients the disappearance from the plasma was followed during the first 15 min which demonstrated that the radiocholesterol was cleared from the circulation with half-life of approximately 10 min. In the same patient the distribution of radioactivity in lipoproteins was estimated by lipoprotein electrophoresis followed by localization of radioactivity on the strip. In every case the distribution conformed to that of cholesterol in healthy controls (4).

Table I Clinical data for eight patients with radiolucent gallstones and two controls

MW=molecular weight, R=limits of control values of the laboratory

Case no.	Age (y)	Sex	Height (cm)	Weight (kg)	% of ideal weight	Plasma cholesterol (mg/100 ml) MW 387 R 140-300	Plasma triglycerides (mg/100 ml) MW 885 R 35-160	History
Patients								
1	73	♀	160	80	136	263	130	Myocardial infarction
2	64	♀	161	85	142	283	?	Cerebral thrombosis
3	65	♀	165	80	129	263	239	
4	62	♂	171	81	119	263	124	Gastric resection
5	73	♀	155	75	134	383	204	Myocardial infarction
6	48	♂	167	68	105	279	124	
7	72	♀	153	82	152	194	106	Cerebral thrombosis
8	45	♀	170	60	91	232	168	
Controls								
1	43	♂	182	78	104	286	?	
2	37	♂	180	71	96	309	?	

Pasting plasma samples were obtained first twice, later once weekly from the patients in the course of the first 10 weeks after injection of the tracer. Plasma cholesterol was determined according to Abeil (10). Plasma triglycerides were analysed by the Boehringer Mannheim UV test. Radioactivity was measured on the hexane extract by liquid scintillation counting. All analyses were carried out in duplicate.

Calculations The results were analysed as described by Goodman and Noble (5) in terms of a 2-pool model (Fig. 1). The specific radioactivity of plasma cholesterol was plotted in a semilogarithmic system against time. In all cases the plots conformed well to a 2-pool model, with a curved part during the first 4-5 weeks, and a linear plot and this time (Fig. 2). The two lines used for the portion of the curve in two exponentials were drawn, the kinetic parameters were calculated according to

Goodman and Noble (5). By assuming that the removal rate of cholesterol from pool B to outside the system (k_B) approximates zero it was possible to calculate the rate constants for transfer between the two compartments (k_{AB} and k_{BA}), and for removal of cholesterol from pool A to outside the system (k_A). This assumption has been evaluated by several authors (5, 6, 20) and is generally accepted.

The results were subjected to statistical analysis by means of Mann-Whitney's rank sum test (21).

RESULTS

The parameters of cholesterol kinetics are given in Table II and Fig. 3. In view of the few controls in our study the results were compared with those from Sandhofer et al (15) who used essentially the same method.

The statistical analysis failed to demonstrate significant differences ($p=0.1$) between any of the parameters from the gallstone patients and the compiled controls.

DISCUSSION AND CONCLUSION

Some limitations of the 2-pool method have to be considered before making comparisons with other results and drawing final conclusions.

Firstly it has been shown by long-term studies that the cholesterol decay curve can be better de-

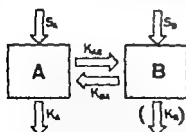


Fig. 1 Model for kinetics in 2-compartment system (9). A=the rapidly exchangeable pool, B=the slowly exchangeable pool. S_A , S_B =rate of entry of cholesterol into pools A and B. k_A , k_B =rate constants for outputs from pools A and B. k_{AB} , k_{BA} =rate constants for transfer between the two compartments. The parentheses indicate the assumption, that output from pool B takes place only through pool A.

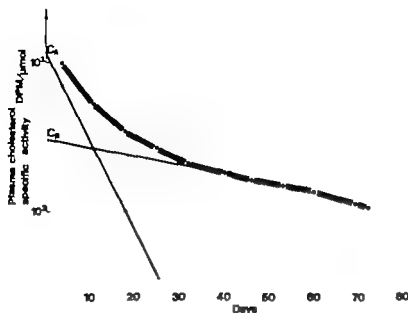


Fig. 2 Decay curve of the specific activity of plasma cholesterol in patient 6. The straight lines illustrate the resolution of the curve into two exponentials.

Table II. Kinetic data of plasma cholesterol turnover in eight patients with radiolucet gallstones and 16 controls from another study

α =fractional turnover rate of the rapidly miscible pool A, β =fractional turnover rate of the slowly miscible pool B, k_{AA} =rate constant for the total removal from pool A, k_{AB} =rate constant for the total removal from pool B, M =size of pool A, PR_A =rate of input into pool A, k_{BA} =rate constant for transfer from pool B to pool A, k =rate constant for removal from pool A excluding transfer to pool B (size of cholesterol from pool A in outside the system), k_{AB} =rate constant for transfer from pool A to pool B

Cate no.	α day ⁻¹	β day ⁻¹	k_{AA} day	k_{AB} day ⁻¹	M (mg/kg)	PR _A (mg/day/kg)	k_{BA} day ⁻¹	k day ⁻¹	k_{AB} day ⁻¹
Patients									
1	0.1284	0.0141	-0.1041	-0.0384	330	15.5	0.0384	0.0471	0.0570
2	0.1690	0.0141	-0.1273	-0.0516	279	12.6	0.0516	0.0451	0.0824
3	0.1540	0.0134	-0.1203	-0.0491	340	16.4	0.0491	0.0483	0.0720
4	0.1386	0.0164	-0.1010	-0.0540	369	15.6	0.0540	0.0421	0.0589
5	0.1690	0.0164	-0.1348	-0.0466	267	15.3	0.0466	0.0581	0.0767
6	0.1444	0.0139	-0.1155	-0.0428	397	18.6	0.0428	0.0468	0.0687
7	0.1540	0.0141	-0.1305	-0.0376	273	15.7	0.0376	0.0578	0.0727
8	0.1540	0.0169	-0.1212	-0.0497	368	19.3	0.0497	0.0534	0.0698
Mean	0.1504	0.0152	-0.1194	-0.0466	328	16.2	0.0462	0.0497	0.0697
S.E.M.	0.0045	0.0005	0.0043	0.0022	18	0.7	0.0022	0.0021	0.0030
Controls									
1	0.1824	0.0175	-0.1261	-0.0738	374	16.2	0.0738	0.0433	0.0648
2	0.1444	0.0163	-0.1144	-0.0463	369	18.7	0.0463	0.0508	0.0536
16 controls from Sandhofer et al. (15)									
Mean	0.1606	0.0162	-0.1308	-0.0460	326	18.3	0.0460	0.0568	0.0740
Mean of all controls	0.1609	0.0163	-0.1296	-0.0476	331	18.2	0.0476	0.0557	0.0740
S.E.M.	0.0034	0.0008	0.0041	0.0018	8	1.0	0.0018	0.0033	0.0031
P (patients and all controls) ^a									
	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1

^aCalculated by assuming that $k_{AB}=0$
Mann-Whitney rank sum test.

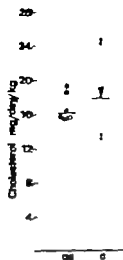


Fig. 3 Input of cholesterol into pool A in eight patients with radiolucent gallstones (gs) and two controls from the present study plus 16 controls from Sandhofer et al. (15) (c). O = results from the present study

scribed by a 3-pool model (6-14). For all practical purposes, estimations of the size of pool A are the same with both methods, but if the third pool is neglected, the input into the rapidly exchangeable pool A will be overestimated by about 8-9% and the size of the slowly exchangeable pool severely underestimated. For this reason we have omitted an estimation of this compartment.

Only Nestel et al. (12) found that compartment analysis tends to overestimate the cholesterol oxidation rate by about 10% in obese individuals compared to the results achieved by means of the sterol balance technique. Since we have used the 2-pool model and since most of our patients were obese, these considerations imply that our results for input into pool A represent maximum values, whereas our estimations of the size of this pool should be valid.

The size of pool A (M_A) in our patients (328 ± 18 mg/kg) was essentially the same as that in the controls (331 ± 8 mg/kg). Using the 3-pool model Hoffman et al. (11) found the size of pool A to be 390 ± 20 mg/kg in eight gallstone patients and were not able to demonstrate differences from results achieved by other authors in patients with various hyperlipidaemias. Consequently it is reasonable to assume that the amount of rapidly exchangeable

cholesterol is within normal limits in patients with cholesterol gallstones. This is of particular interest since observations indicate that biliary cholesterol and de novo synthesized bile acids are derived primarily from this pool (13-20). Thus it is unlikely that the diminished bile acid pool in patients with cholesterol gallstones (17) can be due to a diminished amount of readily available cholesterol.

The input into pool A (PR) was 16.2 ± 0.7 mg/day/kg in the gallstone patients versus 18.2 ± 1.0 mg/day/kg in the controls, the difference being non-significant. To our knowledge only one other group has performed a similar study (11). Using the 3-pool method, the input into pool A was found to be 16.2 ± 0.08 mg/day/kg in eight gallstone patients and no differences were observed from controls collected from the literature. Taken together these results indicate that patients with cholesterol gallstones have normal rates of input of cholesterol into the rapidly exchangeable cholesterol mass.

Grundy et al. (7, 8) using the sterol balance technique, found indications of increased synthesis of cholesterol in American Indian women with gallstones (17.7 mg/day/kg) compared to Indian men without stones (14.3 mg/day/kg). This result might seem to conflict with ours, but it must be stressed that the synthesis of cholesterol is not the same as the input into pool A. The latter parameter comprises both the endogenous synthesis and the absorbed amount of exogenous cholesterol (5, 9). Thus the results of Grundy et al. and our study can be combined in a non-conflicting way if one assumes that the absorption of cholesterol is diminished in gallstone patients. This speculation conforms well to the facts that the absorption of cholesterol is highly dependent upon the presence of bile acids in the small intestine (3) and that the biliary secretion of bile acids is decreased in gallstone patients (7, 8).

Although this indirect suggestion needs confirmation, it does afford the possibility of a more detailed concept of the genesis of gallstone, including the recent emphasis placed by Grundy et al. (7, 8) upon the role of cholesterol.

ACKNOWLEDGEMENTS

The work was supported by grants from Statens Lægevidenskabelige Forskningsråd, Aalborg Kommunes Fond til Medicinsk Forskning and P. Carl Petersens Fond.

REFERENCES

1. Adairand, W. H. & Small D. M. The physicochemical basis of cholesterol gallstone formation in man. *J. clin. Invest.* 47: 1043 1968.
2. Cahlin, E., Jönsson, J., Nilsson, S. & Schersten T. Biliary lipid composition in normolipidemic and probeta hyperlipoproteinaemic gallstone patients. *Scand. J. Gastroint.* 8: 449 1973.
3. Dietschy J. M. & Wilson, J. D. Regulation of cholesterol metabolism. *New Engl. J. Med.* 281: 1179 1970.
4. Dyerberg, J. Lipoproteins in plasma. *FaDL, Copenhagen* 1972.
5. Goodman, D. S. & Noble R. F. Turnover of plasma cholesterol in man. *J. clin. Invest.* 47: 231 1968.
6. Goodman, D. S. Noble R. P. & Dell R. B. Three-pool model of the long-term turnover of plasma cholesterol in man. *J. Lipid Res.* 14: 178 1973.
7. Grundy S. M., Deane W. C., Adler R. D., Aron, J. M. & Metzger A. L. Biliary lipid output in young women with cholesterol gallstones. *Metabolism* 23: 1174 1974.
8. Grundy S. M., Metzger A. L. & Adler R. D. Mechanism of lithogenic bile formation in American Indian women with cholesterol gallstones. *J. clin. Invest.* 51: 3026 1972.
9. Grephe, E., Mass, J. & Sandberg, E. Determination of kinetic parameters in two-pool system by administration of one or more tracers. *Biochem. J.* 125: 1964.
10. Henry R. J. *Clinical chemistry* Harper & Row New York 1964.
11. Hoffmann, N. E., Hofmann, A. F. & Thistle, J. L. Effect of bile acid feeding on cholesterol metabolism in gallstone patients. *Mayo Clin. Proc.* 49: 236, 1974.
12. Nestel, F. J., Schreibman P. H. & Ahrens E. H. Cholesterol metabolism in human obesity. *J. clin. Invest.* 52: 2389 1973.
13. Quarfordt, S. H. & Greenfield M. F. Estimation of cholesterol and bile acid turnover in man by kinetic analysis. *J. clin. Invest.* 52: 1937 1973.
14. Samuel, P. & Peil W. Longterm decay of serum cholesterol radioactivity: body cholesterol metabolism in normals and in patients with hyperlipoproteinaemia and atherosclerosis. *J. clin. Invest.* 49: 346, 1970.
15. Sandhofer E., Bolzano, K., Sailer S. & Braunstein H. Studies on cholesterol turnover in normocholesterolemic subjects. *Europ. J. clin. Invest.* 2: 426 1972.
16. Small, D. M. & Rapo, S. Source of abnormal bile in patients with cholesterol gallstones. *New Engl. J. Med.* 283: 33 1970.
17. Vlahovic, Z. R., Bell, C. C., Bubac, L., Farrar J. T. & Swell, L. Diminished bile acid pool size in patients with gallstones. *Gastroenterology* 59: 165 1970.
18. Vlahovic, Z. R., Bell, C. C., Gregory D. H., Baker G., Justfodden, P. & Swell L. Relationship of bile acid pool size to the formation of lithogenic bile in female Indians of the Southwest. *Gastroenterology* 62: 73 1972.
19. Vlahovic, Z. R., Bell C. C. & Swell L. Significance of the liver in the production of lithogenic bile in man. *Gastroenterology* 59: 62, 1970.
20. Wilson, J. D. The measurement of the exchangeable pools of cholesterol in the baboon. *J. clin. Invest.* 49: 435, 1970.
21. *Wissenschaftliche Tabellen*, p. 124. Gessy Basel 1968.

ANOREXIA NERVOSA AND THE FREQUENCY OF SEX CHROMATIN POSITIVE CELLS

Jan Wållinder

*From the Psychiatric Research Centre, St Jürgen's Hospital, University of Göteborg
Hisings Backa, Sweden*

Abstract Five consecutive cases of anorexia nervosa in women have been investigated for variation in the frequency of sex chromatin-positive cells. The results showed a statistically significant relation between body weight and the frequency of sex chromatin-positive cells, the amount of sex chromatin increasing with rising body weight. These findings are difficult to explain, but the results might conceivably be used at least in some cases as an index of changing basal biological functions in anorexia nervosa.

In recent years papers have been published on the relation between endocrine disorders in women and low frequency of sex chromatin-positive cells. Relations of this type have been recorded both in congenital adrenal hyperplasia (4, 13) and in hypothyroidism (7). It is also known that the frequency of sex chromatin-positive cells tends to decline in relation to a high content of oestrogenic hormone in the blood and that administration of other steroid hormones and ACTH can reduce the frequency of sex chromatin (14, 16). Further, it has been recorded that during the first weeks of infancy the frequency of sex chromatin is low, rising thereafter to the levels found in adult women (15, 16).

During the last ten years a number of reports have appeared concerning the coincidence of anorexia nervosa and Turner's syndrome (karyotype 45 X) in the same woman. Seven such cases have been published (2, 3, 5, 8, 9, 10, 12) and although it is not yet clear to what extent they represent anything more than random coincidence, the coexistence of two such uncommon disorders is certainly worthy of note. It has been suggested that in all cases of anorexia nervosa, complete karyotype analysis is advisable in order not to miss cases of mosaicism.

While a combined case of this type was under study some years ago, it was observed in the hospital genetic laboratory that a girl with anorexia nervosa showed a remarkably low frequency of sex chromatin-positive cells during the anorectic phases of her illness. On suspicion of Turner's syndrome (mosaicism) karyotype analysis was carried out but this proved to be normal. Repeated determinations of sex chromatin revealed a striking rise in conjunction with increased body weight (1). This observation stimulated closer study of the changes in sex chromatin in anorexia nervosa.

MATERIAL AND METHODS

The investigation was carried out upon five consecutive female cases of anorexia nervosa referred to our clinic. Their ages varied from 17 to 34 (mean 27.8). The diagnosis was established according to the following criteria: Anorectic behaviour as defined by Palazoff Selvin (11), i.e. "the conscious and stubborn determination to emaciate one-self despite the presence of an intense interest in food. Additional requirements were: high degree of progressive weight loss, secondary amenorrhoea and other signs of secondary hypogonadism, laboratory evidence of reduced thyroid function without clinical signs of myxoedema and normal distribution of axillary and pubic hair.

On admission to hospital each patient had a buccal smear which was examined for the presence of sex chromatin. The buccal film was fixed in alcohol and stained with toluidine and 900 cells were assessed in every specimen. Repeat test were made every second week during the hospital stay. Simultaneously with each test, all the patients had estimation of total oestrogen in the urine and some of them also had determination of the thyroid functional level and the content of gonadotropins (FSH and LH) in the blood. Treatment for all the patients was standardized and consisted of bed rest for the first two or three weeks, administration of small doses of

Table 1 Frequency of sex chromatin-positive cells (%) related to current body weight (kg)

No of observations	Case 1		Case 2		Case 3		Case 4		Case 5	
	Sex chromatin	B wt.	Sex chromatin	B wt.	Sex chromatin	B wt.	Sex chromatin	B wt.	Sex chromatin	B wt.
1	20	38.9	23	41.7	11	30.4	20	68.5	20	36.4
2	24	40.9	26	44.1	33	34.9	19	67.5	22	38.5
3	29	42.7	32	45.5	31	36.0	22	68.6	29	39.1
4	33	45.5	33	46.2	28	39.1	24	73.0	28	41.2
5	29	46.4			33	39.0	29	73.3	28	43.4
6	39*	54.6*			34	39.5	35	73.9	33	44.0
7					28	40.5	28	75.0	31	42.1
8					30	43.5	32	75.5	32	42.8
9									32	40.4
10									29	42.0
11									34	41.9
<i>p</i>	0.93 <0.01		0.96 <0.05		0.61 N.S.		0.87 <0.01		0.78 <0.01	

* Recorded 5 months after the 5th observation.

chlorpromazine (max. 75 mg daily) and carefully graduated increase of caloric intake. At first this was mainly in the form of fluid nutrients, which were subsequently progressively replaced by normal diet. The investigations were discontinued when the patients had increased so much in weight and were in such physical and mental condition that they were considered fit for discharge or when they requested this. At the time of discharge none of the patients had had return of menstrual periods.

RESULTS

Table 1 shows the frequency of sex chromatin-positive cells in the five patients as determined in successive tests set in relation to body weight time of the tests. The number of observations varied from a maximum of 11 to a minimum of 4 observations per patient (mean 7.4). It is clear from the Table that in every case there was a strong correlation between body weight and the frequency of sex chromatin, the amount of the latter rising with increasing weight. The correlation coefficients varied between 0.61 and 0.93. Testing by means of Fisher's χ^2 -transformation showed that all but one of the figures were statistically significant. The mean value of the correlation coefficients was similarly determined by means of Fischer's z transformation and amounted to 0.82 ($p < 0.001$). Homogeneity testing by means of χ^2 -analysis carried out on the transformed correlation coefficients showed no significant deviation of any of the recorded figures from the rest.

Table I does not include data from the hormonal analyses. In general these showed no correlation either with body weight or with the frequency of sex chromatin, they did not vary significantly during the investigation period and above all showed no definite increasing tendency. Only in one case (no. 4) was it noted that as body weight increased the oestrogen output in the urine became normal. It should be mentioned that the weight of this patient on admission was as much as 68.5 kg. However this was about 10–12 kg below her normal weight and in other respects her condition was one of undoubted anorexia nervosa.

DISCUSSION

In my material the amount of sex chromatin varied with the body weight: a rise in weight being associated with a statistically significant increase in the frequency of sex chromatin. The frequency of sex chromatin-positive cells varied from a minimum of 15% to a maximum of 39%. Most of these figures lie within the normal range of variation for sex chromatin frequency as reported by Smith et al. (15) (19–49%) and by Hamerton (6) (36–76%).

The findings of this study are difficult to interpret. It has already been mentioned that the frequency of sex chromatin is influenced by hormonal factors. It has also been observed that extensive burns cause a drastic fall of sex chromatin during the acute stage of the injury (17). In anorexia nervosa

sa, reduced levels of oestrogenic hormones are found in the blood and urine, as well as low levels of gonadotropic hormones (FSH and LH). Previous observations indicate that high oestrogen content in the blood correlates with low sex chromatin frequency and my findings therefore cannot be explained on these grounds. It has also been shown that administration of adrenal cortical steroids or ACTH similarly induces a fall in the frequency of sex chromatin. In anorexia nervosa however there is a low urinary output of corticosteroids and the levels of plasma cortisol are normal. There is therefore no parallel in this respect either. On the other hand in anorexia nervosa there is a reduction of metabolism as measured for example by the BMR or other tests of thyroid function. As mentioned above low frequency of sex chromatin is found in hypothyroidism and this finding is therefore in line with my observations.

Weight gain is not the only sign of improvement in a patient with anorexia nervosa. There is a rise in such biological variables as basal temperature, pulse rate and blood pressure and these often precede recovery before there is any change in weight. The present investigation suggests that change in the frequency of sex chromatin-positive cells is another such variable and that at least in some cases it can serve as an index of improvement in basal biological functions.

REFERENCES

1. Akerson, H. O. Personal communication.
2. Dickens, J. A., *Brit. J. Psychiat.* 117: 237 1970.
3. Foreman, H., McEble, G. & Wilsdor, J. *Brit. J. Psychiat.* 116: 221 1970.
4. Garza, F. J., Asper, A. C. & Smith, D. W., *Lancet* 2: 373 1971.
5. Hales, K. A. & Riggs, C., *Brit. J. Psychiat.* 122: 79 1973.
6. Hamerton, J. L., In: *Human cytogenetics*, vol. 1, p. 133. Academic Press, New York and London 1971.
7. Hochmair, D. & Wierker, D., *Lancet* 2: 978, 1971.
8. Kihlborn, M., *Acta paedopsychiat.* 36: 75 1969.
9. Lindsten, J., The nature and origin of X chromosome aberrations in Turner's syndrome. Almqvist & Wiksell Stockholm 1963.
10. Lison, E. & Shershow, L. E., *Arch. gen. psychiat.* 29: 834 1973.
11. Peliccioli Schvini, M., In: *Anorexia nervosa* (ed. J. E. Meyer and H. Feldman), p. 96. Thieme Stuttgart 1965.
12. Pitts, F. N. & Garza, S. B., *Amer. J. Psychiat.* 119: 1100 1963.
13. Schneer, J. H. & Nagel, A., *Lancet* 2: 1089 1971.
14. Shetty, K. T., Sharma, N. L. & Wehal, K. M., *Brit. med. J.* 2: 84 1966.
15. Smith, D. W., Marden, P. M., McDonald, M. J. & Speckhard, M., *Pediatrics* 90: 707 1962.
16. Tylor, A., *Lancet* 1: 912, 1963.
17. Wass, S., Barnett, J. S., Garson, O. M. & Baile, A. G., *Lancet* 1: 745 1967.

Announcements

The Pio Istituto di S Spirito ed Ospedali Riuniti Roma In collaboration with the Accademia Lancisiana di Roma announces a competition for the 1975 *G M Lancisi International Prize for Medical Science* of Lit. 10 000 000 to be assigned to the author(s) of an original unpublished scientific work dealing with the subject *Clinical Immunology*

Those intending to enter for the prize should send the following to Premio Internazionale Scientifico G M Lancisi presso la Presidenza del Pio Istituto S Spirito - Borgo S Spirito 3 I-00193 Roma Italy by Dec 31 1975

1) Application on plain paper to participate in the competition 2) Nine copies of the work entered for the prize in Italian or in English, each copy bearing the signature of the author(s) All works accompanied by positive copies of any illustrations should include a synopsis in Italian or English of not more than 1 000 words as well as a full bibliography of the subject dealt with. 3) Certificates testifying to the birth residence and citizenship of the competitor(s) 4) A copy of the curriculum vitae and of the scientific activity of the competitor(s) with a list of published works 5) A declaration signed by the author(s) testifying to the original nature of the work the institute or other place where the work has been carried out that said work is unpublished and that it has not been submitted and will not be submitted until such time as the G M Lancisi prize been assigned to other prize competitions.

International Heparin Symposium will be held in London July 18-19 1975

Further information Mr V V Kakkar Department of Surgery King's College Hospital Denmark Hill London SE5 8RX England

The XII Congress of the European Dialysis and Transplant Association (E.D.T.A.) will be held at the Copenhagen Bella-Center Aug. 26-29 1975 The official languages of the congress are English and French

Further information E.D.T.A. Congress DIS-Congress Service Knabrostræde 3 DK 1210 Copenhagen K Denmark.

International Colloquy on Lipoproteins and Hyperlipidemias will be held in Lisbon, Portugal Sept. 3-6 1975

Further information. Prof. M. J. Halpern, Dept. of Biochemistry Faculty of Medicine of Campo Santana, Lisbon Portugal

2nd International Meeting of Medical Advisers in the Pharmaceutical Industry will be held in Palazzo dei Congressi Florence Italy Oct. 13-15 1975

Further information Organizing Committee Secretariat Dr N. Bergamini Dr V. Bachini Via R. Lepetit 8 Milano Italy

TOTAL DISAPPEARANCE OF A FATAL PULMONARY EMBOLUS DURING STREPTOKINASE THERAPY OF AN ILIOFEMORAL THROMBOSIS

Jan Eklund Evald Johansson and Christer Paul

From the Departments of Anaesthesia and Surgery and the Coagulation Laboratory Karolinska Hospital Stockholm, Sweden

Abstract During streptokinase treatment of an iliofemoral thrombosis, a formerly healthy man developed clinical signs of major pulmonary embolism. 1 split of all at attempts at resuscitation the patient died after 1.5 hours. The autopsy showed no signs of remaining thrombus or emboli. The probable explanation is that the thrombus had partially been lysed and that the remaining parts formed an embolus large enough to cause considerable haemodynamic changes which could not be influenced. Lysis continued pre- and postmortally and was complete at the time of autopsy.

For many years the relationships between clinical and pathological findings have been well established. Most often the findings at autopsy can be predicted and sometimes the clinical picture is so characteristic that an autopsy seems almost unnecessary. However, therapy changes and it should not be forgotten that the introduction of new methods may completely change a morphological picture familiar to a pathologist. Having met with such a situation in the unsuccessful treatment of a man with a deep venous thrombosis who died from a classical pulmonary embolism, we found it of particular interest to report the features of this case.

CASE REPORT

A formerly healthy and quite alert 77-year-old man was hospitalized because of pain and swelling of the right leg for two days. The obvious clinical diagnosis was an iliofemoral thrombosis. Ascending phlebography showed an almost total occlusion of the veins up to the inferior vena cava. Apart from peripheral thrombocyte count of 56 000 and a bleeding time of 12 min there were no abnormalities on laboratory examination at this time. Active thrombolytic treatment was started by constant infusion of streptokinase (Kabikines® Kabi, Stockholm, Sweden). Initial dose (4000 IU) was performed instead the patient received 300 000 IU streptokinase during one

hour after which the treatment continued with 100 000 IU/hour.

Four hours after treatment started the thrombin time was more than double the normal indicating a fibrinolytic effect. After 15 hours treatment the patient was definitely improved. He had no pain and only moderate swelling of the leg remained. No complications to the thrombolytic therapy had been observed. Two hours later the patient suddenly deteriorated with all the classical signs of a major pulmonary embolism, including circulatory collapse.

The patient was immediately treated with oxygen and assisted ventilation and was rapidly transferred to the Intensive Care Unit. Further he received papaverine (80 mg), morphine (50 mg), theophylline (200 mg) i.v. and isoprenaline (1 mg) i 300 ml of 5.5% dextrose solution. Nevertheless he did not improve and because of cyanosis he was intubated and ventilated with pure oxygen and positive end-expiratory pressure of 10 cm H₂O by means of an Engström ventilator 300 (LKB-medical, Stockholm, Sweden) without any evident improvement.

During this treatment the ECG showed gradually increasing signs of myocardial hypoxia, but no arrhythmias were observed. The systolic BP varied between 90 and 80 mmHg. After one hour treatment the circulation failed completely though the ECG showed fairly regular rhythm. No pulses could be felt and the pupils became dilated. External cardiac compression was without effect and at no time could any sufficient circulation be obtained. The resuscitation was stopped after 1.5 hours.

Four hours after death the body was transferred to a cold room and the autopsy was performed 96 hours later. At this time neither pulmonary embolus nor deep venous thrombus could be found. The heart was dilated but not hypertrophic and there were signs of venous congestion in both lungs and the liver. No signs of myocardial infarction could be found. Multiple bleeding was observed in the pancreas.

DISCUSSION

As there had been no doubt about the clinical diagnosis of major pulmonary embolism, the post mortem findings came as a complete surprise.

However on further consideration we have accepted the following explanation

Before the treatment started the patient had a thrombosis engaging the main veins of the entire right leg. As he improved markedly it must be assumed that most of the thrombus had been dissolved. However enough material was left to constitute a considerable embolus which unfortunately was lethal even though it was under continuous dissolution. If the embolus had not totally disintegrated at the time of death, it is quite possible that lysis continued during the first hours afterwards. Consequently no solid material was found at autopsy. The tragic fact that the patient succumbed in spite of successful thrombolysis was probably due to the persisting haemodynamic changes signs of which were to be seen at autopsy. These changes together with hypoxia and—most certainly—a considerable stress were evidently too great a strain for this fairly old man.

The hypothesis of a continuous and finally total lysis of a lethal embolus is supported by the findings of Gajewski (1). In his report of a lethal case of

pulmonary embolus during thrombolytic therapy he described the embolic material as gelatinous half liquid and thus partially lysed.

As active fibrinolytic therapy is being used more and more in patients with thromboembolic disease (2, 3, 4, 5) we find it important to point out this possible change in a formerly distinct pathology.

REFERENCES

- 1 Gajewski J. Thrombolytic therapy and fatal massive pulmonary emboli. *Ann. Intern. Med.* 74: 450, 1971.
- 2 Kakkar V. V., Flanc C., O'Shea M. J., Flete P. T., Howe C. T. & Clarke M. B. Treatment of deep-vein thrombosis with streptokinase. *Brit. J. Surg.* 56: 178, 1969.
- 3 Mevor G. E., Dhall D. P., Dawson, A., Duthie J. S. & Walker M. G. Streptokinase in deep vein thrombosis. *Brit. J. Surg.* 60: 468, 1973.
- 4 Robertson, B. R.: On thrombosis, thrombolysis and fibrinolysis. *Acta Ch. scand. Suppl.* 471, 1971.
- 5 Tsapogian, M. J., Peabody R. A., W. K. T., Karmody A. M., Devanaj K. T. & Eckert C.. Controlled study of thrombolytic therapy in deep vein thrombosis. *Surgery* 74: 973, 1973.

OVARIAN CYSTS IN WOMEN ON CHRONIC INTERMITTENT HAEMODIALYSIS

Jørn Hess Thaysen Klaus Øigaard and
Herluf G. Jensen

From Medical Department P, Division of Nephrology, Rigshospitalet, Copenhagen, Denmark

Abstract In consecutive material of 42 female patients, who were treated with intermittent dialysis for chronic renal failure between May 1964 and Nov 1971, 1 developed ovarian cysts. The cysts were found only among the 29 women, who had menstrual periods from commencement of dialysis (16 cases) or who started menstruating on dialysis after period of secondary amenorrhoea (13 cases). No cysts were found in the remaining 13 patients, who had amenorrhoea throughout, and of whom 6 were all probability postmenopausal. Seven of the 21 patients with ovarian cysts had pronounced symptoms, necessitating acute surgery in 4. Fourteen asymptomatic cases were diagnosed at routine gynaecological examinations, which was performed at regular intervals in all patients. There is strong evidence suggesting that the development of ovarian cysts was somehow related to the dialytic treatment and neither to the aetiotic state nor to the nature of the primary renal disease. The mechanisms by which dialytic treatment may be operational in the development of this hitherto undescribed complication is discussed, but no clear-cut explanation can be given.

In 1967 a 20-year-old woman, who had been on chronic intermittent haemodialysis for 39 months, was acutely admitted because of severe abdominal pain. Emergency laparotomy was performed and a multicystic torquated left ovarian cyst containing haemorrhagic fluid was removed. Histological examination showed a follicle cyst with haemorrhage. The right ovary was slightly enlarged with small cysts on the surface. One month later the patient was readmitted with an identical clinical picture. Acute surgery disclosed a rupture of an 8 × 8 cm large cyst in the right ovary with haemorrhage into the peritoneal cavity and an oophorectomy was performed. The histological diagnosis was a "simple cyst".

This serious event led to a systematic prospective investigation of the occurrence of ovarian cysts in

women on chronic intermittent haemodialysis and a surprisingly high frequency of ovarian cysts was found. It is the purpose of the present paper to discuss the nature of this complication, which does not appear to have been reported before in the literature.

MATERIAL

Patients on chronic intermittent dialysis

The material consists of 42 consecutive female patients who were taken onto chronic haemodialysis between May 1964 and Nov 1971.

Renal disease. The diagnosis of renal disease was established by conventional clinical and radiological criteria supplemented by histological examination of renal tissue obtained by biopsy and/or at bilateral nephrectomy. With due reservation for the well known fact, that a histological diagnosis may be difficult in unusually contracted kidneys, the diagnoses were as follows: chronic glomerulonephritis 16, chronic interstitial nephropathy 16 (largely comprising cases with analgic abuse), polycystic kidney disease 4 and in the remaining 6, nephrosclerosis, thrombotic thrombocytopenic purpura, Alport disease, medullary sponge kidney, congenital renal abnormality and bilateral traumatic renal lesions.

Age at start of dialysis averaged 40 years (range 17-59).

Menstrual periods. Six of the 42 women, average age 46 years (range 43-50), had amenorrhoea before and during dialysis, and were all probability postmenopausal. Among the remaining 36 women, average age 38 years (range 17-49), 16 had menstrual periods before and during dialysis, 13 with secondary amenorrhoea resumed normal menstrual period following an average of 3 months on dialysis (range 1-10), whereas 7 remained amenorrhoeic throughout. Thus, 29 of the 36 premenopausal women had menstrual period on dialysis.

Control material

The control material consisted of 27 randomly selected non-dialysed women with severe renal failure. Endogenous creatinine clearance averaged 11 ml/min (range 4-20).

Renal disease The diagnosis of renal disease was established by clinical criteria supplemented by renal biopsy. The diagnoses were: chronic interstitial nephropathy (mostly due to analgesic abuse) 16, chronic glomerulonephritis 9, polycystic kidney disease 1 and nephrosclerosis 1.

Age at gynaecological examination ranged 41 years (range 20-55).

Menstrual period Among the 77 women 15 (56%) had regular menstrual periods. Of the remaining 11 amenorrhoeic women 6 (average age 47 years) were in all probability postmenopausal.

METHODS

Dialysis procedure The patients were dialysed with Gambro or Gambro-Lundia dialyzers for an average period of 10 hours (range 9-11) twice a week. They received a diet containing on an average 0.8 g protein/kg b.wt. and sodium, potassium and fluid intake was restricted according to urinary losses. No patient had sustained hypertension. Tumors had been bilaterally nephrectomized during dialysis.

The patients were heparinized during dialysis by single injections of heparin. The return-line from the dialyzer under repeated control of clotting time, which varied from 15 to 60 min. The total dose of heparin per dialysis ranged from 15 000 to 35 000 IU. Apart from occasional increases in the plasma flow and duration of menstrual periods, other haemorrhagic complication were exceedingly rare.

Gynaecological examination of the patient on chronic haemodialysis was performed at start of treatment at regular intervals during treatment (at least every 6 months) and if presence of symptoms. In the control material only one examination was carried out.

RESULTS

In the control material of 77 severely uremic women ovaries in 1 were found.

In the 4 haemodialysed patients no ovarian cysts were diagnosed. At the first examination at the start of treatment.

However, in the course of chronic haemodialysis this diagnosis was made in 1 of the 4 women. Based on the way the diagnosis was made these 1 patients were divided into 3 groups (Table I).

Group I 4 cases. Operation because of ruptured ovarian cyst. In all cases the histological diagnosis was follicle cysts or "simple cyst" with haemorrhage.

Group II 3 cases. Acute abdominal pain and the finding of ovarian cysts at the gynaecological examination. The complaint disappeared on conservative treatment.

Group III 14 cases. These patients had no symp-

oms and the diagnosis was made at the regular gynaecological examinations.

The following case histories are typical examples of the clinical course in the three groups.

CASE REPORTS

Case 1 group I

A 37-year-old woman with chronic glomerulonephritis, admitted in June 1969 in terminal renal failure. The patient was dialysed twice a week for 14 months (143 dialyses). In Oct. 1970 she was transplanted with necrotic kidney. Two months later she was discharged with an endogenous creatinine clearance of 40 ml/min.

Gynaecological data Menarche at the age of 11. She had always been regularly menstruated without delays, abortions or gynaecological complaints. She had never been treated with oestrogens or progesterone. At start of the dialysis treatment gynaecological examination was normal. During dialysis the menstrual period continued but the individual periods were stronger and of longer duration. During the 38th haemodialysis treatment (4 months after start) the patient developed severe abdominal pain.

At laparotomy a 5 x 5 cm large ruptured cyst in the right ovary and about 700 ml blood in the peritoneal cavity were found. A partial resection of the ovary was performed. Histological examination showed simple cyst. One month later—in the middle of a menstrual period—an identical clinical picture developed during the 45th haemodialysis treatment. At gynaecological examination a 7-7 cm large cyst in the left ovary was found and a left salpingo-oophorectomy was performed. The ovary contained non-ruptured cyst of the above mentioned size. Histological examination showed follicle cyst with haemorrhage.

Case 2 group II

A 26-year-old woman, admitted in Oct. 1968 in terminal uraemia due to chronic glomerulonephritis.

In July 1969 after 9 months of haemodialysis, kidney transplantation was performed with kidney from the patient's brother (A-match). The postoperative course was without complication and the patient is still doing well with an endogenous creatinine clearance of 60 ml/min, normal BP and protein-free urine.

Gynaecological data Menarche at the age of 13. Regularly menstruated until 1 month before the above mentioned admission when the patient became amenorrhoeic. She had no delays or abortions, and no previous gynaecological complaints. She had never been treated with sex hormones. Gynaecological examination at the start of haemodialysis treatment was normal. One month after the beginning of the haemodialysis treatment the patient became regularly menstruated. The day after the 16th haemodialysis treatment (2 months after start) the patient had pain in the right iliac fossa. She was in the middle of menstrual period and gynaecological examination showed an enlarged cystic right ovary. The pain disappeared on conservative treatment. During the following 6 months normal conditions were found at bi-monthly

Table 1 Gynaecological data in 42 women on chronic intermittent haemodialysis

	Groups with ovarian cysts			Total	Without ovarian cysts
	I	II	III		
N of patients	4	3	18	21	21
Duration of dialysis period (mo.)	31 (14-39)	17 (7-38)	13 (1-31)	17 (1-39)	5 (1-27)
Age at start of dialysis (y.)	31 (17-45)	31 (24-46)	35 (18-49)	33 (17-49)	40 (28-50)
Duration of dialysis until signs of ovarian cysts (mo.)	20 (4-32)	18 (2-35)	7 (1-18)	10 (1-35)	
Regular menstrual periods throughout (no. of pts.)	3		7	1	4
Secondary amenorrhoea with resumption of periods during dialysis (no. of pts.)	1	1	7	9	4
Amenorrhoea throughout					13
Hormonal treatment during dialysis period			6	6	3

gynaecological examinations. One week before transplantation the right ovary was again cystically enlarged but the patient had no subjective complaints. Gynaecological examinations 3 and 18 months after transplantation were normal.

Case 3 group III

A 36-year-old woman admitted in May 1969 in terminal oedema due to chronic glomerulonephritis.

After 12 months of chronic intermittent haemodialysis the patient received a nephrectomy. Due to thrombosis of the renal artery the allograft was removed 5 days after transplantation and the patient returned to chronic intermittent haemodialysis, the total period of dialysis being 29 months.

Gynaecological data. Menarche at the age of 14. Always regularly menstruated. Two normal deliveries, 1959 and 1961. In 1968 an abortion was performed due to the chronic kidney disease. No sex hormone therapy was given. Gynaecological examination at the beginning of the haemodialysis treatment was normal. Routinely performed gynaecological examination 6 months after the start of dialysis (i.e. after 48 dialyses) showed an ovarian cyst on the left side. This cyst was found at later gynaecological examinations but was of varying size. After 28 months of treatment (corresponding to 224 dialyses) large cyst was found in the right ovary.

The patients with ovarian cysts were encountered in all types of renal disease. There was no preponderance in patients with polycystic kidneys.

All 41 patients with ovarian cysts were menstruating when the diagnosis was made. Among the 21 women without ovarian cysts only 8 were menstruating during the dialysis period.

The duration of the dialysis period was on an average 17 months in the group with ovarian cysts and 5 months in the group without cysts.

In all patients in groups I and II (Table 1) the abdominal pain arose during the haemodialysis procedure or on the following day. Six of the 11 patients with ovarian cysts and 3 of the 8 menstruating patients without ovarian cysts were for short periods treated with oestrogens and/or progesterone largely for contraceptive purposes.

Twelve of the 41 women with ovarian cysts were transplanted, all but one with nephrectomy. Four died shortly after the transplantation but in the remaining 8 patients no ovarian cysts were found at gynaecological examinations performed 7-4 months after grafting.

DISCUSSION

At laparotomy Varo and Niemineva (3) demonstrated dysfunctional ovarian cysts with a frequency of about 7% in a large material. The incidence of symptomatic ovarian cysts is in all probability much lower although we have not been able to find exact figures pertinent to this question.

In the present material 21 of 42 consecutive female patients on chronic intermittent dialysis had ovarian cysts demonstrated either at regular gynaecological examination or by symptoms. The frequency of asymptomatic and symptomatic cysts was 1/42 (50%) in all women, 1/36 (58%) in the premenopausal women and 1/9 (77%) in the menstruating women. The frequency of symptomatic cysts was 7/41 (17%) in the whole material, 7/36 (70%) in the premenopausal women and 7/29 (4%) in the menstruating women. This frequency is so high that a further investigation of a possible rela-

tion to the primary renal disease to the uremic state and to the dialytic procedure seemed relevant.

With respect to the primary renal disease it is pertinent that the presence of cysts was randomly distributed between patients with different kidney diseases. Twenty of the 47 women were bilaterally nephrectomized but there was no preponderance of cystic disease of the ovaries in the nephrectomized group. As regards the possible role of the uraemic state per se the following findings seem to be relevant.

1) No patients in a control material of 77 severely uraemic but non-dialysed women had ovarian cysts.

2) No cysts were found at routine gynaecological examination at commencement of dialytic therapy in the 42 dialysed patients.

3) In 11 patients, in whom cysts were demonstrated during dialysis and who were successfully transplanted, the cysts could not be demonstrated at follow-up examination after grafting.

The above mentioned findings indicate that the development of cysts is related neither to the underlying renal disease nor to total nephrectomy nor to the uraemic intoxication per se. The evidence that the development of cysts is, somehow related to the dialysis treatment therefore appears convincing.

Concerning the pathogenetic mechanism for the development of ovarian cysts in haemodialysed women two factors may be considered: 1) The effect of heparinization.

2) A hormonal imbalance. A regards heparinization, it is striking that aggravation of symptoms in the seven symptomatic cases did always occur either during the haemodialytic procedure or shortly afterwards. In the six instances where surgical intervention was deemed necessary (two women being operated on twice) fresh haemorrhage was always found in the cysts. There is thus little doubt that heparinization may aggravate symptoms by causing haemorrhage into preformed cysts but is highly unlikely that heparinization may be operational in the development of cysts. Despite a careful perusal of the literature we have found no mention of the development of ovarian cyst in menstruating women who were anticoagulated for reasons other than haemodialysis.

As regards the possible role of a hormonal imbalance somehow induced by haemodialysis, it is worth mentioning that all 11 cases of ovarian cysts developed among the 79 patients who were

menstruating during dialysis either from commencement of treatment (16 cases) or after a period of secondary amenorrhoea (13 cases). No ovarian cysts were observed in the remaining 13 women, of whom 11 were in all probability postmenopausal whereas 7 may represent cases of secondary amenorrhoea not relieved by dialysis.

It must be of interest to know whether the menstruating women had ovulatory cycles or not. In our opinion there is considerable evidence in favour of anovulatory cycles: 1) Measurements of basal temperature in a few of the patients of the present material as well as in a study of haemodialysed menstruating women since 1971 indicates that the cycles are anovulatory. 2) Pregnancy is exceedingly rare in haemodialysed premenopausal women. 3) Measurements of plasma follicle-stimulating (FSH) and luteinizing hormones (LH) (see below) also favour the hypothesis of anovulatory cycles.

We found that the clearance of plasma oestradiol in conventional dialyzers is exceedingly low usually less than 1 ml/min of the non-protein-bound fraction. This finding taken together with the measurements of plasma FSH and LH indicates that depletion of oestradiol and/or progesterone from the plasma during haemodialysis resulting in an increased secretion of FSH and LH is hardly of significance.

Digaard et al. (1) found plasma FSH and plasma LH values within normal limits but without cyclic variation in premenopausal women dialysed in our department since 1971. The FSH/LH ratio was however high and not low as found by Abraham et al. (1) in otherwise normal women with anovulatory cycles. Whether this relatively high FSH/LH ratio can explain the development of cysts in our patients is uncertain.

The histological studies of the ovarian cysts removed in six ovaries in four of our patients showing either follicle cysts or "simple cysts" is of no aid in understanding the mechanism of the development of ovarian cysts.

We are thus still in complete ignorance about the pathogenesis of ovarian cysts in our haemodialysed women. The chief purpose of the present paper is therefore to present this hitherto undescribed complication to haemodialysis in order to see whether other treatment centres can retrospectively or prospectively verify its existence. If so a much more careful investigation about the pathogenetic mechanism becomes highly relevant.

REFERENCES

1. Abraham, G. E., Odell, W. D., Swerdloff, R. S. & Hopper, K. Simultaneous radioimmunoassay of plasma FSH, LH, progesterone, 17-hydroxyprogesterone and estradiol-17 during the menstrual cycle. *J. clin. Endocr.* 34: 312, 1972.
2. Olgaard, K., Hagen, C. & McNelly, A. S. The pituitary hormones in women with chronic renal failure. The effect of chronic haemodialysis and peritoneal dialysis. Submitted to *Acta endocr. (Kbh.)*.
3. Vana, P. & Niemelä, K. Small-cystic degeneration of ovaries as an incidental finding in gynecological laparotomies. *Acta obstet. gynec. scand.* 31: 94, 1952.

Medical Journals

Printed and distributed by Almqvist & Wiksell
publishers to the Universities of Uppsala, Stockholm and Göteborg
and to the Royal Swedish Academy of Science, etc.

Acta Chirurgica Scandinavica

Editor: L. Thoren

8 issues per volume. Free supplements. Including free subscriptions to the *Scandinavian Journal of Plastic and Reconstructive Surgery* (without suppl.), the *Scandinavian Journal of Thoracic and Cardiovascular Surgery* (without suppl.) and the *Scandinavian Journal of Urology and Nephrology* (without suppl.)

Current volume 141/1975

Sw kr 290 per volume, incl. postage

Acta Dermato Venereologica

Editor: Nils Thyrenon

6 issues per volume. Free supplements.

Current volume 55/1975

Sw kr 130 per volume, incl. postage

Acta Medica Scandinavica

Editor: J. Waldenström

6 issues per volume. Free supplements.

Current volumes 197-198/1975

Sw kr 225 per annum (two volumes), incl. postage

Acta Obstetrica et Gynecologica Scandinavica

Editor: Axel Ingelman-Sandberg

5 issues per volume. Free supplements.

Current volume 54/1975

Sw kr 150 per volume, incl. postage

Acta Oto Laryngologica

Editor: C. A. Hamberger

6 issues per volume. Free supplements.

Current volumes 79-80/1975

Sw kr 100 per volume. Two volumes per annum

Sw kr 200, incl. postage

Pædiatrica Scandinavica

✕ R. Zetterström

issues per volume. Free supplements.

Current volume 64/1975

Sw kr 175 per volume, incl. postage

International Journal of Fertility

Editor: S. J. Behrman

4 issues per volume

Current volume 20/1975

Sw kr 120 per volume, incl. postage

International Journal of Gynecology and Obstetrics

Editor: Harold A. Kaminitzky

6 issues per volume.

Current volume 13/1975

Sw kr 110 per volume, incl. postage

Scandinavian Audiology

Editor: Björn Blegvad

4 issues per volume. Free supplements.

Current volume 4/1975.

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Infectious Diseases

Editors: Jostia Siron and Sten Wimbliad

4 issues per volume. Free supplements.

Current volume 7/1975.

Sw kr 110 per volume, incl. postage

Scandinavian Journal of Plastic and Reconstructive Surgery

Editor: Bengt Johanson

3 issues per volume. Free supplements.

Current volume 9/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Psychology

Editor: Lars Kebabon

4 issues per volume.

Current volume 16/1975

Sw kr 98 per volume incl. postage

Scandinavian Journal of Rehabilitation Medicine

Editor: Oile Höök

4 issues per volume. Free supplements.

Current volume 7/1975

Sw kr 90 per volume, incl. postage

Scandinavian Journal of Rheumatology

Editor: Veikko Laine

4 issues per volume. Free supplements.

Current volume 4/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Social Medicine

Editor: Gunnar Inghe

3 issues per volume. Free supplements.

Current volume 3/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Thoracic and Cardiovascular Surgery

Editor: Viking Olov Björk

3 issues per volume. Free supplements.

Current volume 9/1975

Sw kr 100 per volume, incl. postage

Scandinavian Journal of Urology and Nephrology

Editor: Åke Fritjofsson

3 issues per volume. Free supplements.

Current volume 9/1975.

Sw kr 100 per volume, incl. postage

Uppsala Journal of Medical Sciences

Editor: Gunnar Ågren

3 issues per volume. Current volume 80/1975

Sw kr 70 per volume, incl. postage

Free inspection copies on request—write to

The Almqvist & Wiksell Periodical Company
Box 62, S 101 20 Stockholm Sweden

EPIDEMIOLOGY OF RENAL STONES IN A MIDDLE AGED MALE POPULATION

Sverker Ljunghall and Hans Hedstrand

From the Department of Internal Medicine, University Hospital, Uppsala, Sweden

Abstract. The natural history of upper urinary tract stones has been studied retrospectively in 49-50-year-old men in an urban population. The prevalence of stones in 2322 men was found to be 13.7% with the highest incidence of onset of the disease during the fifth decade. Recurrences had occurred in 42% of all cases, the frequency increasing with the observation time. On some occasions 23% of the patients had been admitted to hospital and 12.3% had been operated on, 94.5% of all stones passed spontaneously. A family history of kidney stones was significantly more common in stone patients than in healthy controls, and patients with family history of stones were more prone to early and repeated recurrences. It is suggested that the raised incidence of stone disease in some families may be attributed to environmental rather than genetic factors. This could be of importance for prophylaxis. Analysis of hospital admission rates supported previous findings of a steady rise in stone incidence. The advantages of population studies for comparative analyses are pointed out.

The incidence of urinary tract stone disease has never been established precisely in any but the smallest communities (20). Comparative analyses of hospital admission rates indicate considerable temporal variations, with a steady rise in the incidence of upper urinary tract stones during this century (3) and marked geographic variations (3-6). Such differences may be attributed to ethnic, socio-economic or nutritional factors (3) but there is a need for further information on dietary, climatic and racial influences in the occurrence of urinary tract stone (7). The natural history of renal stones in patients admitted to hospital has been the subject of thorough investigations and these reveal among other things a high recurrence rate (24-29). Hospital admission rates can however be influenced by factors other than the incidence of the disease, and cases examined in hospital may not fully represent uncomplicated stone disease. It was therefore considered worthwhile to perform an epidemiologic

study of stone disease in the population. Such a study might also disclose groups with divergent clinical pictures, e.g. with a high recurrence rate where further investigations could provide additional information on the etiology of renal stones.

This study reports a survey of five consecutive age classes of middle-aged men in whom the incidence and natural history of stones in the upper urinary tract have been investigated.

MATERIAL AND METHODS

During 1970-73, health examination survey was offered all men living in the city of Uppsala, Sweden, who were born in 1920-24 (1). A total of 2322 men were examined, a participation rate of 83.9%. This investigation included a detailed self-administered questionnaire according to Collen et al. (8). One question concerned kidney stones and read: "Have you ever suffered from renal colic?" All patients who answered affirmatively were recalled for detailed interview. Only patients with history of stones in the upper urinary tract were included but no effort was made to distinguish between kidney and prostatic stones. The term kidney stones is, in this paper, used synonymously with upper urinary tract stones.

Only patients who at the interview fulfilled at least one of the following four criteria were accepted for the present study: 1) Spontaneous passage of stone noticed by the patient. 2) Operative removal of kidney or prostatic stones. 3) Roentgenologically demonstrated stones of the upper urinary tract. 4) Characteristic clinical findings judged by physician at the time of symptoms. The patient's statements about his symptoms were accepted in those cases included according to criterion 4 even when records from the examining physician were not available.

The clinical history was broken into separate units called "incidents" (29). Each incident represented stone or group of stones with continuous symptoms. If symptoms were not terminated by observed passage of stone, all symptoms during the following year were attributed to the same incident. Also succession of stones passed at short intervals without roentgenological evidence of stone-free urinary tract in between was classified as only one incident. After symptom-free period of one year

Table 1 Total number of stones in 318 middle-aged men in relation to the age at onset of symptoms

Age at onset (y)	Total no. of stones							Total no. of pairs	
	1	3	4	5	6-9	10		n	%
18	1							3	0.9
20-4	5	1	3			3		16	5.0
5-9	6	6		1	3	3		25	7.9
10-14	7	4	3		2	4		24	7.6
15-19	39	18	4		1	6		73	23.0
20-24	3	13	10	3		5		64	20.1
25-29	74	6	6		1			91	28.6
Total no. of pairs	185	49	3	16	6	9	1	318	100.0

new symptoms were considered to be due to the formation of a new stone even if indisputable evidence of stone passage had not been produced. In the text the term "stone" generally denotes the concept of "incident".

All data concerning operations were collected from the original hospital record. Cystoscopes for purely diagnostic purposes were not included, nor were unsuccessful attempts at endoscopic stone extraction. Serum calcium and uric acid estimations and qualitative test for cystine in urine (Brand test) were performed in almost all cases. Detailed investigations for diagnosis of stone etiology were carried out only when relevant to the clinical picture e.g. in patients with many recurrences.

Information regarding the occurrence of kidney stones among first-degree relatives was obtained through postal questionnaire to all stone patients. The same questionnaire was also sent to control group consisting of all other men in the city born in 1974, reported to be stone-free at the health survey. 309 stone patients (98.1%) and 393 controls (91.5%) replied to the inquiry. This information was accepted without further attempts of confirmation. A family history of kidney stones was considered to exist if one first-degree relative had had a kidney stone even if the informant did not have full knowledge of all relatives. On the other hand absence of family history of stones was accepted only if the informant could negate the occurrence of stones in all his first-degree relatives. Consequently if he did not possess information concerning all relatives, he was not included in the calculations regarding family occurrence of stones. Adequate information was given by 269 stone patients (83.5%) and 330 controls (74.5%).

With the aid of postal questionnaires, a small study was made of the occurrence of kidney stones among the non-participants in the health survey. Of 66 individuals born in 1974 who had not taken part in the examination, 49 answered this enquiry. This information was also accepted without further investigation.

Patient data concerning in-patient care in the city of Uppsala during 1969 was collected with the National Social Welfare Board's permission, through discharge information on each individual from the University Institute of Social Medicine.

Student's *t*-test, the χ^2 -test with Yates correction for small populations, and Fischer exact test for 2 tables were used in the statistical analyses. Accepted level of significance was $p < 0.05$.

RESULTS

Stone prevalence

Of the 318 men aged 49-50 who participated in the health survey, 343 (14.8%) answered the question: "Have you ever suffered from renal colic?" affirmatively. This could not be substantiated at the following interview in 25 cases of which 11 were not available for investigation. In 9 cases the diagnosis was questionable and 5 individuals had mistakenly acknowledged that they had had renal colic. The remaining 318 men represent 13.7% of the investigated male population in these age groups. In 136 cases the diagnosis was considered verified by observed passage of stones, operation or X-ray (criteria 1-3) on at least one occasion. In the remaining 8 individuals the diagnosis was regarded as highly probable even though no stone had been visualized (criterion 4). Of these patients 73 had a single stone and thus in only 9 patients with recurrences were stones never visualized.

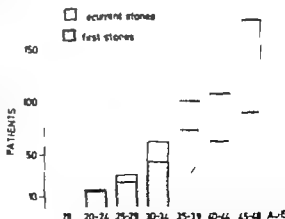


Fig. 1 Total number of patients with kidney stones during each 5-year period. No patient is included more than once during each period.

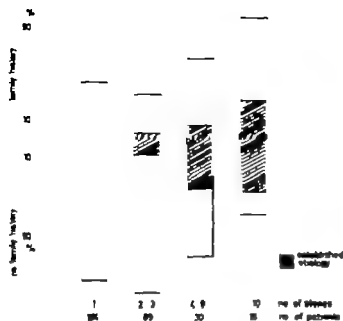


Fig. 2 Family history of kidney stones in relation to number of stones. Frequency of recognized etiology of stone formation in relation to family history and number of stones. Only patients with full information regarding stone disease in relatives are included.

Among the non-participants in the health survey who answered a postal enquiry 20.4% reported kidney stones. This figure is not significantly different from that of the participants.

Table I shows that kidney stones were rare before the age of 20 but after 30 the number of stone patients rapidly increased. This increase continued up to the age of 50. The number of patients with recurrences also increased with time. The highest stone incidence consequently occurred in the 5-year period preceding the health survey when 179 individuals had at least one stone which corresponds to 7.7% of all men in these age groups (Fig. 1).

Possible etiology of stone formation

Although it was beyond the scope of this study to establish an etiology of stone formation in every instance a possible cause was found in 30 cases (9.1%) mainly patients with several recurrences. Among the diagnoses were nine operatively verified cases of hyperparathyroidism, three cystinurias and three medullary sponge kidneys.

Family history of stones

Kidney stones in at least one first-degree relative (family history of stones) were present in 29.4% of stone patients and 15.3% of stone-free control individuals from the same health survey. This difference is highly significant ($p < 0.001$). A family history of stones was particularly common in patients with

multiple stones (≥ 4). Fig. 2 shows that this raised incidence of family history in multiple stone formers came entirely from patients with no known cause of stone formation whereas if possible diagnosis was established a family history of stones was not more common in patients with recurrences.

Recurrences

Altogether 133 patients (42%) had experienced at least one recurrence of stone by 50 years of age. The risk of relapse increased continuously from 31.5% after 5 years to 72% in patients who had their first stone attack more than 20 years previously.

Patients with a family history of kidney stones had on an average experienced more stones by the age of 50 than those without stones among relatives. This could not be explained by any difference in age at the onset of stone disease, nor was the total number of patients with recurrences significantly different (Table II). However multiple recurrences were significantly more common in patients with a family history of stones and the early recurrence rate in these patients was significantly increased. Whether previous stones had been operatively removed or passed spontaneously did not seem to influence the recurrence rate.

Medical care

This material derives from a city population with good access to emergency medical care and with

Table II Comparison between patients with and without family history of kidney stones with regard to age at onset of symptoms, total number of patients with recurrences and frequency of multiple and early recurrences

Only patients with full information regarding stone disease in relatives are included

	Age at onset (y)	Total recurrence rate (%)	≥3 recurrence (%)	Recurrence (%)	
				In 5 y	In 10 y
Family history (n=79)	37.9	46.9	27.9	48.2	46.8
No family history (n=190)	38.3	41.2	12.6	25.8	32.4
Significance of difference	ns	ns	p<0.01	p<0.001	p<0.001

ns=not significant.

local traditions favouring early hospital attendance in case of acute symptoms. The medical care for these patients is presented in Table III. In cases without apparent passage of stone, a physician's diagnosis was required if the patient was to be included in the present study. This is however a minor reason for the high rate of medical care, since very few persons were omitted for not fulfilling this criterion.

The proportion of patients treated at hospital on some occasion increased with the number of stones and only a small number of patients with repeated recurrences were treated exclusively in private practice. It was at the same time evident that patients with experience of stones from several recurrences attended medical care less often, particularly in cases of early spontaneous passage of the stone.

Operation

In all 811 stones were reported, 47 stones (5.5%) in 39 patients (12.3%) were removed at operation. Spontaneous stone passage thus terminated 94.5% of the stone incidents. The number of operations is too small to permit any detailed separation according to surgical technique. Half of the operations

consisted of ureteral surgery almost exclusively ureterolithotomies. Operations on the kidneys and endoscopic stone extractions were equally common and made up the other half.

There was a tendency for the operation rate to increase with age. Before the age of 40, 3.8% of stones were removed at operation compared to 6.9% after 40 years of age. Another finding was a lower incidence of operations in patients with multiple stones (≥4). In these patients only .8% of all stones required operation, whereas 9.2% of patients with fewer stones were operated on.

Hospital statistics

In order to obtain an estimate of the development of the incidence of kidney stones in this region, statistics for the two hospitals serving the city of Uppsala were analysed for 1969. During this year a total number of 138 patients of both sexes were admitted to hospital because of stones in the upper urinary tract. This represents 83 individuals per 100 000 population. The age distribution and the proportion of operations for the men in this hospital material are compared in Fig. 3 with the information given by the participants in the health survey. This comparison shows a substantial increase in the hospital admission rate in the younger age groups. Among 25-9-year-old men, hospital admissions during 1969 were five times more common than among the participants in the health survey at the corresponding age. The figure also shows a continually increasing rates with age for hospital admissions and operations for the men in the health survey. During 1969, however, hospital admissions for upper urinary tract stones were equally common in the age groups 35-9 and 45-49 for males in Uppsala.

Table III Medical care for kidney stones in male stone patients in an urban population

	%
Admitted to hospital at least once	3.0
Hospital out-patients, never admitted	55.0
Physicians outside hospital only	17.9
No medical care	4.1
	100.0

DISCUSSION

As far as accuracy in diagnosis is concerned there are obvious advantages associated with only investigating patients admitted to hospital. However for epidemiological purposes less rigid criteria for diagnosis sometimes have to be accepted. A considerable proportion of ureteric stones verified by X-ray pass spontaneously shortly after the onset of symptoms without apparent stone passage (24). It was therefore considered justified to include symptomatic cases without definitely visualized stones in this investigation. These cases constitute about 25% of the total, mainly patients with single stones. This proportion between objectively verified and mainly symptomatic cases agrees fairly well with other reports concerning clinically typical cases of stones in the upper urinary tract (9, 16, 24).

In judging the natural history primarily from information given by the patients there are possibilities of over as well as underestimating recurrences. Since the majority of stones in the ureter pass within weeks of the onset of symptoms (10, 24) and in this investigation repeated symptoms within one year were attributed to the same stone, the risk of introducing false positive recurrences seems small but some early recurrences (within the first year) may not have been recognized. A recent investigation has shown that the number of completely asymptomatic stones is probably small and that the value of repeated X-ray control for their discovery is limited (22). Most recurrences are therefore believed to have been properly noticed by the patients.

Comparisons between compilations of hospital admissions for urinary tract calculi in different parts of Sweden (11) and other parts of Europe and the

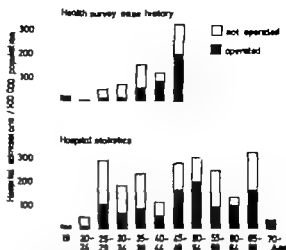


Fig. 3. Hospital admissions for kidney and ureteric calculi in men in Uppsala. Case history information given by the participants in the health survey and hospital statistics from 1969. Numbers are expressed per 100 000 population in the respective age groups.

USA show large variations, with a rather low hospital admission rate in the province of Uppsala (21). Compared to other population studies the present prevalence of kidney stones in middle-aged men (13.7%) does not support the presumption that the low hospital admission rate reflects a low stone incidence (Table IV). In fact, only 23% of these stone patients have on any occasion been admitted to hospital in spite of excellent facilities for such admissions. This clearly demonstrates the difficulty in using hospital admission rates as a measure of the incidence of this disease. The results from the three health surveys of middle-aged men in the different parts of Sweden presented in the Table indicate

Table IV. Prevalence of upper urinary tract stones in men in population studies (diagnosis based on clinical picture)

Country	Group	Prevalence (%)	Reference
Denmark	Doctors, all ages	12	(16)
USA	All ages (estimated figure)	4.2	(23)
Great Britain	Royal Navy 50-year-olds (estimated figure)	3.5	(4)
Norway (Oslo)	50-59-year-olds	11.6	(27)
Sweden			
Gothenburg	Health survey 50-year-olds (only visualized stones)	6.8	(25)
Malmö	Health survey 55-year-olds	4.1	(13, 14)
Uppsala	Health survey 49-50-year-olds	13.7	Present study

however that local circumstances may considerably influence the incidence of kidney stones.

The rapid increase reported previously (3) for the incidence of stones in the upper urinary tract seems to be continuing. Since earlier population studies are lacking, these comparisons have to be based on hospital admission rates. Hedenberg (11) in an analysis of hospital statistics from the province of Uppsala, found an annual incidence of stones in the upper urinary tract of 19 per 100 000 inhabitants during 1943-46. The present study of the same hospitals 15 years later shows a five-fold increase. The principles for admission do not appear to have changed at these hospitals in recent years and this increase therefore may reflect a true rise in stone incidence. The rise in incidence over time and the possibilities of large geographic variations have to be considered in analyses of stone occurrence e.g. in the selection of all sorts of control materials.

In analyses of cross-sections of the population it is often found that the commonest age at onset of stone disease is during the third or fourth decade and a decline is common in middle age (1, 2, 4, 5, 17, 18, 29). However it is at the same time apparent that for the men in this study the incidence increased continually over the years and any decline would have to occur after the age of 50. This rising incidence during life may be related to aging as such but also to prolonged exposure to environmental factors. If the latter are predominant and of increasing importance, younger age groups will be affected to a greater extent. This may explain why the incidence is about the same in the different adult age groups in the population as a whole when the risk for the individual is rising so markedly.

In this study more than one eighth of all men had been affected by kidney stones by the age of 40. The number of recurrences also increased with time and of patients with a disease of more than 20 years duration 77% formed another stone. Thus the formation of kidney stones is not a singular event but a process in which the risk of recurrence remains for many years. Given enough time most patients seem to relapse. Large series have also failed to distinguish between patients who are apt to have recurrent stones and those who are not. As far as blockage of the urinary tract is concerned, the incidence of infection is the same in patients with stones as in those without. The only difference is that patients with stones are more likely to have a urinary tract infection. Apart from the fact that the etiology of the disease is different, the treatment is the same for kidney stones as for urinary tract infections.

Patients with an increased risk of early and frequent recurrences partly because in these cases prophylactic measures may be indicated and partly in order to gain a better understanding of idiopathic stone disease. In this study the patients with a family history of stone disease from an epidemiological point of view constituted such a group with a greatly increased risk.

The observed co-variation between a family history of stones and the clinical picture with repeated recurrences may indicate that patients with stones among their relatives have features in common which make them liable to stone formation. Only a few rare causes of stone formation are definitely known to be inherited and probably constitute too small a number to significantly affect the calculations in this study. That a family history of kidney stones is more common in stone patients than in control individuals has been shown before (17, 19, 23) and is confirmed in the present study. It has previously been considered unlikely that the data in these respects are significantly biased by patients with stone disease having a heightened interest in stones amongst their relatives (19, 23). Whether a familial occurrence is due mainly to genetic or environmental influence is not known. Resnick et al. (23) found evidence compatible with the hypothesis that the tendency to form calcium oxalate stones is regulated by a polygenic system. However Transbol and Frydendal (26) suggested that environmental factors might be involved and be responsible for their finding of a higher percentage of affected male relatives among patients with idiopathic hypercalciuria than when no metabolic cause for stone formation was discovered. The importance of non-genetic household factors was also stressed by White et al. (28) who found an increased urinary calcium excretion in spouses of stone-formers.

Though some inherited predisposition may also be involved, it seems that environmental factors at least in some instances can contribute to the formation of stones. Epidemiological studies might be useful for further work on the identification of such factors in the environment, the ultimate goal being to provide a rational prophylaxis and treatment of idiopathic stone formation.

ACKNOWLEDGEMENT

This study was supported by a grant from Skandia Insurance Company, Stockholm, Sweden.

REFERENCES

- 1 Ahlgren, S. A. & Löfstad M. Renal and ureteral calculi in Swedish district. *Acta chir. scand.* 130:344 1965
- 2 Alken, C. E. & Hermann, G. E. Untersuchungen über die Urolithiasis unter besondere Berücksichtigung der Bevölkerungsstatistik. *Urol. int.* 4 315 1957
- 3 Andersen H. A. Historical and geographical differences in the pattern of incidence of urinary stones considered in relation to possible aetiological factors. In: *Renal Stone Research Symposium* (ed. A. Hodgkinson and B. E. C. Norda). Churchill London 1969
- 4 Blacklock, N. J. The pattern of urolithiasis in the Royal Navy. In: *Renal Stone Research Symposium* (ed. A. Hodgkinson and B. E. C. Norda). Churchill, London 1969
- 5 Boukauer K. Morphologie und Genese der Harnsteine. In: *Handbuch der Urologie* (ed. C. E. Alken, V. W. Di. K. M. Weymann and E. Wildbois). Springer Berlin 1961
- 6 Boyce, W. J. Garvey F. K. & Strawstetter H. E. Incidence of urinary calculi among patients treated in general hospitals 1948-1952. *J.A.M.A.* 161 1437 1956
- 7 Clark P. B. Hodgkinson A. Norda B. E. C. & Wilkerson, R. E. Foreword in: *Renal Stone Research Symposium* (ed. A. Hodgkinson and B. E. C. Norda). Churchill London 1969
- 8 Cohen, M. F. Cutler J. L., Siegelman A. H. & Cella, R. L. Reliability of self-administered medical questionnaire. *Arch. intern. Med.* 123 664 1969
- 9 Frank, M. de Vries A. Atsman, A. Latebrik, J. & Kozma, S. Epidemiological investigation of urolithiasis in Israel. *J. Urol.* 81 497 1959
- 10 Gottfries, A. Holmquist, B. Idoborn, H. von Porat, B. & Wikander C. Har ofta uppstår irreversibla skador på njuren vid uratersten och hur skall dessa komma undvikas? *Läkartidningen* 71 2186, 1974
- 11 Hedberg, I. Renal and ureteral calculi: study of the occurrence Sweden during 1911-1938 with some notes of the geographical distribution. *Acta chir. scand.* 101 17 1951
- 12 Hedstrand H. A study of middle-aged men with particular reference to risk factors for cardiovascular disease. *Ups. J. Med. Sci.* In press 1975
- 13 Isaacson, S. O. Venous occlusion plethysmography in 55-year old men. A population study in Malmö Sweden. *Acta med. scand. Suppl.* 537 1972.
- 14 — Personal communication. 1974
- 15 Koltwitz, A. A., Kracht, H. Bräuer R. & Löbe E. Klinische und Laborbefunde bei 470 Patienten mit Kalziumsteinen der oberen Harnwege. *Urol. int.* 24 318, 1969
- 16 Larsen, J. F. & Philip J. Studies on the incidence of urolithiasis. *Urol. int.* 13 III 1962.
- 17 Lavan, J. N. Neale F. S. & Poser, S. U. nary calculi, clinical, biochemical and radiological studies in 619 patients. *Med. J. Aust.* 2 1049 1972.
- 18 Mates, J. & Krizek, V. Die Steinkrankheit im Lichte von 3340 beobachteten Fällen. *Z. Urol.* 48 478 1955
- 19 McGowan, M. Hereditary renal stone disease. *Clin. Sci.* 19 465 1960.
- 20 Norda B. E. C. Metabolic bone and stone disease. Churchill Livingstone, Edinburgh and London 1973
- 21 Norda B. E. C. & Hodgkinson A. Urolithiasis. *Advanc. intern. Med.* 13 155 1967
- 22 Powis, S. J. Black, J. Macdonald J. A. & Clews, J. W. Management of patient with urinary calculi. *Brit. med. J.* 1 355 1974
- 23 Resnick, M. Pringle H. B. & Goodman H. O. Genetic predisposition to formation of calcium oxalate renal calculi. *New Engl. J. Med.* 278 1313 1968.
- 24 Sandegård, E. The prognosis of the stone in the ureter. *Acta chir. scand. Suppl.* 219 1956
- 25 Tibbén, G. High blood pressure in men aged 50—a population study of men born 1913. *Acta med. scand., Suppl.* 470 1967
- 26 Tammblom I. & Frydendal, N. Endocrine and metabolic aspects of urology. Aetiology of stone formation in 145 renal stone patients. *Acta chir. scand. Suppl.* 433 137 1973
- 27 Westlund K. Urolithiasis and coronary heart disease. A note on association. *Amer. J. Epidemiol.* 97 167 1973
- 28 White, R. W. Cohen, R. D. Vince, F. P. Williams, H. Blandy J. & Tremblay C. G. Minerals in the urine of stone-formers and their spouses. I. *Renal Stone Research Symposium* (ed. A. Hodgkinson and B. E. C. Norda). Churchill London 1969
- 29 Williams, R. E. Long-term survey of 538 patients with upper urinary tract stone. *Brit. J. Urol.* 35 416 1963

ADULT TYPE OF POLYCYSTIC KIDNEY DISEASE IN A NEW-BORN CHILD

Ulla Bengtsson, Lars Hedman and Christian Svalander

From Medical Department I, Renal Unit and Department of Pathology, Sahlgren Hospital,
University of Göteborg, Göteborg, Sweden

Abstract. A case of polycystic kidney disease in a new-born child is reported. Renal cortical necrosis due to asphyxia was the cause of death. The histopathological picture and heavy family history support the rare diagnosis of polycystic kidney disease of adult type in a new-born child.

Polycystic disease of the new-born or infant is represented by a variety of gross and microscopic pictures. Most children with polycystic disease are still-born or die soon after parturition. On rare occasions polycystic kidney disease has been diagnosed in early childhood in families with the dominantly inherited adult form of polycystic kidneys (1-5, 6).

The following is a presentation of a new-born child with polycystic kidneys. The adult type of polycystic kidneys had been manifest in four earlier generations of the family.

CASE HISTORY

A 22-year-old woman had right-sided cystadenoma simplex resected during the 4th month of her first pregnancy. Two weeks before full term, in Dec. 1972, she was admitted to hospital because of slight proteinuria. Her BP was normal. Twelve days later she developed toxemia, the fetal heart rate decreased, and caesarean section was carried out on the indication of impending asphyxia. On exploration of the mother's abdomen polycystic kidneys were found. The liver was normal on palpation. The post-operative course was uneventful.

The new-born was a girl, mature and with no external malformations. She remained asphyctic and was taken to ward for intensive care. She had edema, passed no urine, and was found to be uraemic on the following day. Peritoneal dialysis was started, but the child died two days after birth. At autopsy polycystic kidneys and cortical necrosis were found (Figs. 1-4).

Family history

The mother had 5 years earlier had an attack of left-sided flank pain and microscopic hematuria. Urography showed

a small contrast defect in the left kidney and the episode was judged to be due to a passage of concretions. The kidneys were normal-sized and not even in retrospect were there any signs of cysts. A year after parturition she has undergone a new urography which shows enlarged kidneys, length 18 cm, and the appearance of cysts of varying size. Her glomerular filtration rate is normal, but her concentration capacity is moderately reduced.

The mother has sisters, 27 and 19 years of age. They are healthy so far but have not been examined. The grandmother had polycystic kidneys diagnosed at the age of 40, developed anemia at 47, when she got renal transplant which is still functioning after 5 years. The grandmother's brother had polycystic kidneys and died from uremia at the age of 45.

Another 11 members of the family are known to have had polycystic kidneys: the great grandmother, 2 great aunts, great-uncle, the grandmother, grandfather and cousin. They all died from anemia between 40 and 50 years of age. Thus, at least 5 generations have been affected by this heritable disease. The family pedigree is shown in Fig. 5.

Pathology

The necropsy showed a left kidney slightly enlarged and right kidney of normal size, total weight 30 g after fixation in 10% formalin. After stripping off the fibrous capsule, multiple cysts, the size of pinhead and containing transparent fluid, were exposed on the cortical surface (Fig. 1). In sections the cortex was mottled brown and yellow and engorged with blood; the medulla was pale with well formed papillae (Fig. 2). There were no malformations of the pelvis, ureters, bladder, urethra or the genital organs. There were no cysts to be found in the liver, which was of normal size, structure and consistency. No congenital malformations were demonstrated in other internal organs. Extensive atelectases of the lungs were found bilaterally; furthermore there were signs of general circulatory disturbance with generalized edema and acute blood stains.

The microscopical examination of the kidneys demonstrated bilateral cortical necrosis of irregular extension and with prominent interstitial haemorrhage from blood-filled capillaries. There were small spherical cysts, usually 1-2 mm in diameter but generally much smaller, almost exclusively localized in the cortex and considera-



Fig 1 The kidneys with capsules removed. Small cysts, hardly visible, are present in the cortical surface. Note the normal extent of fetal lobulation.

by more numerous than could be macroscopically imagined. The distribution was characterized by groups of 5–10 cysts within the cortex (the extension corresponding to groups of neighbouring septulae, and interspaced by normal parenchyma (Fig. 3). Many of the smaller cysts contained a peripherally located glomerulus (Fig. 4). There were no signs of dysplasia (dysontogenetic tissue) in the kidney sections studied.

COMMENTS

At the turn of the century Köster (8) showed that the age distribution of polycystic kidney disease has two peaks: one at the time of birth and one between

40 and 50 years of age (Fig. 6). The bimodality in age at diagnosis has been apparent whenever extensive series of cases have been collected and it has led to the view that there are two distinct entities.

The polycystic kidneys in adults have been well defined from clinical, pathological and genetic viewpoints (3–5). The heredity is autosomal dominant.

Polycystic kidney disease in new-borns and infants is not a homogenous pathological entity and there has been some confusion about the terminology and classification (2, 4–10). Clinical pathologi-

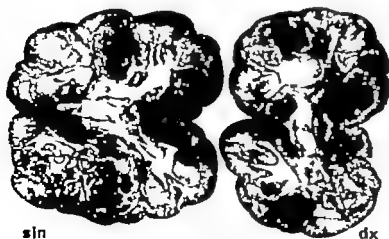


Fig 2 Longitudinal sections through the kidneys. Cortical cysts are hardly visible at this magnification.



Fig 3 Characteristic finding of small collection of cysts in the cortex. The surrounding parenchyma is necrotic (fix-van Gieson $\times 50$).

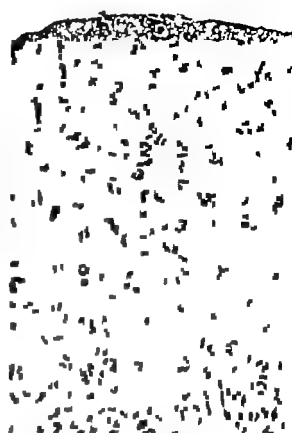


Fig 4 Corpuscular type of cysts with glomerular structures visible in the lumen. Note also the necrotic tubular epithelium especially in proximal tubules (fix-van Gieson $\times 40$).

cal and genetic features have not always been correlated. Thus Osathanondh and Potter classified exclusively on the basis of microdissection studies. In a critical survey Bernstein (1) emphasizes that renal cysts in syndromes of multiple malformations and hypoplastic kidneys with cyst formation should not be confounded with infantile polycystic kidney disease. The most typical pathological picture shows radially arranged fusiform or cylindrical cysts and these changes are usually combined with cysts of the liver (7). This variety seems to be transmitted as a recessive trait manifested only in homozygotes. Siblings are often affected but never the parent.

Most studies on polycystic disease seem to confirm the concept that the entities in childhood differ from that in adulthood. Thus Lundin and Olsson (2) in their study of 23 newborn infants and 11 children found none with family history in earlier generations. There are very few reports of polycystic

kidney disease in early childhood in families with adult type of polycystic kidney. One of these reports suggests the coincidence of an infantile type (associated with hepatic fibrosis) and the adult type in the same family (6). Ferguson (5) was the first to describe a child 5 year-old with polycystic kidneys in family with dominant inheritance of the disease. Blyth and Cockenden (2) described an interesting family with polycystic kidneys in a father and a 2-year-old girl. The mother had a later pregnancy terminated in the 14th week and twin fetuses were found. Microscopic examination revealed few cortical and medullary cystic changes in both kidneys and both livers.

There are many theories of the pathogenesis of polycystic kidney but the causes of both adult and infantile form are still unknown. The cysts in polycystic kidney disease originate from any point along the nephron (7). When they arise from cystic

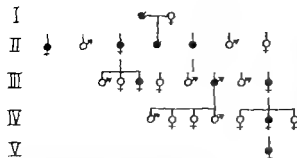


Fig 5 Family Pedigree. ● = polycystic kidneys. Six of the individuals in the 4th generation (aged 19–30) have not been subjected to renal examination.

dilatation of Bowman's capsule glomerular tufts are recognizable in the lumen. Typical for the adult type is the spherical configuration of the cysts and in the earlier stages groups of cysts are surrounded by parenchyma of normal structure.

The new-born child in the present report demonstrated a histopathological picture which might well be compatible with an early stage of adult type of polycystic kidneys. With the heavy family history there can be no doubt about the classification of the kidney disease.

The child died from anuria, which could hardly be due to the cystic disease. The cortical necrosis initiated by an intra and extrauterine asphyxia, seems to be the probable cause. Very little is known about the early development of polycystic kidneys of adult type since clinical signs and symptoms are late manifestation. Only autopsies of fetuses, new-borns and infants from affected families can provide information when death occurs in intercurrent diseases.



Fig 6 Polycystic kidney disease. Age distribution in 39 cases (Köster).

REFERENCES

- Bernstein J. Heritable cystic disorders of the kidney. *Pediatr. Clin. N. Amer.* 18: 435 (1971).
- Blyth II & Ockenden, G. Polycystic disease of kidneys and liver presenting in childhood. *J. med. Genet.* 8: 257 (1971).
- Dalgaard O. Z. Bilateral polycystic disease. Follow-up of 284 patients. *Acta med. scand., Suppl.* 328 (1957).
- Fauré C., Les maladies kystiques des reins chez l'enfant. *Radiol. Clin. Biol.* 41: 212 (1972).
- Ferguson, J. D. Observations on familial polycystic disease of the kidney. *Proc. roy. Soc. Med.* 42: 806, (1949).
- Hoefel J.-C. Jacotot II & Bourgeois, J.-M. A propos d'une famille associant des cas de polykystose rénale de type juvénile et de type adulte. *Ann. Radiol.* 14: 205 (1971).
- Krimke J. M. Congenital malformations. In: *Pathology of the kidney* (ed. R. H. Heptinstall), p. 311. Little Brown and Co., Boston 1966.
- Krüster E. Die chirurgischen Krankheiten der Niere. *Dtsch. Z. Chir.* 52B: 512 (1898–1902).
- Leodi P. M. & Otow I. Polycystic kidneys in newborns, infants and children. A clinical and pathologic study. *Acta paediat. scand.* 50: 185 (1961).
- Opsthaasoodh, V. & Porter E. L. Pathogenesis of polycystic kidneys: I II III IV. *Arch. Path.* 77: 459 (1964).

THE EFFECT OF SPIRONOLACTONE (ALDACTONE®) AND METHYLDOPA IN LOW AND NORMAL RENIN HYPERTENSION

S B Solheim J A Sundsfjord and L. Giezendanner

From the Medical Department Ørskov H. spital Orkanger the Hormone and Isotope Laboratory Aker H. spital Oslo, and the Office for Biostatistics Bergen, Norway

Abstract. The effect on BP of 100 and 200 mg spironolactone/day has been compared with that of methyldopa, 750 mg/day and with combined treatment with both drugs in 32 patients with essential hypertension. The 28 patients who completed the entire investigation were treated for 30 weeks, divided into 4 treatment periods and 4 placebo periods of equal duration. BP did not fall significantly during the initial placebo period and at the end of each of the intervening placebo periods it rose to pretreatment levels. A significant decrease in mean BP was found during the 4 treatment periods. A fall exceeding 14% was registered in 32% of the patients after methyldopa 750 mg/day in 40% of the patients after spironolactone 200 mg/day in 89% after combined treatment with both drugs, and in 29% after spironolactone, 100 mg/day. Low renin hypertension was found in 9 of the 28 patients. The average decrease in mean BP after spironolactone 200 mg/day methyldopa 750 mg/day and after combined treatment did not differ significantly between the low and the normal renin group. The rationale for using diuretics such as spironolactone or thiazide as the basic therapy in essential hypertension is discussed. It is concluded that both are useful in the treatment of essential hypertension and might be used alone or in combination.

Diuretics of the thiazide group are commonly accepted and widely used as a basic therapy in the long-term treatment of hypertension, although these drugs may have profound metabolic side effects which have to be considered when they are used for several decades in subjectively healthy people with clinically diagnosed hypertension. Recent investigations have shown that hypertensive patients may be grouped after their plasma renin activity (PRA) and that whereas spironolactone does control the BP in low PRA hypertension, this is not so certain in normal and high renin hypertension (4, 6, 11, 14, 18, 19).

The two main purposes of the present investiga-

tion have been: 1) To study the effect of spironolactone and methyldopa, alone and in combination and compared with placebo in the treatment of benign essential hypertension. 2) To study the effect of treatment in relation to levels of PRA.

MATERIAL AND METHODS

Fifteen women and 17 men, aged 33-73 years, with the clinical diagnosis of benign essential hypertension were included in this study. All of them had normal renal function and fundus hypertonus grade I or II. BP was measured each time ambulant under as identical conditions as possible with the same adjusted Hg-sphygmomanometer, first after 10 min bed rest, then after 2 min of standing and finally after 10 knee-bendings, and the averages of three measurements being used. The mean BP is defined as diastolic BP + 1/3 of the pulse pressure.

The entire investigation lasted for 30 weeks, each treatment period of 4 weeks being followed in principle by 4 weeks of placebo (Fig. 1). There were 4 treatment periods altogether and a 4-week placebo regimen concluded the study. The majority of the patients had not received antihypertensive treatment previously and in the remaining cases the hypotensive treatment was cancelled 3-4 weeks prior to the study.

Three months after this experiment had been completed, after cancelling hypotensive therapy for the last 2 weeks, blood samples were taken for the determination of PRA (21). The patients had been on normal diets and the samples were taken ambulant, the first sample with the patient in a upright position having walked around for two hours, and the second sample 2 hours after 80 mg furosemide orally. The upper normal level with the present PRA method is 1.3 ng ang I/ml/h, based on ambulatory measurement in more than 600 patients with benign essential hypertension without previous treatment (to be published). Patients with a PRA level of 0.2 ng ang I/ml/h or less before and after stimulation with furosemide were classified as low renin hypertension patients. Plasma aldosterone concentration was determined (22); the low renin group only included primary aldosteronism.

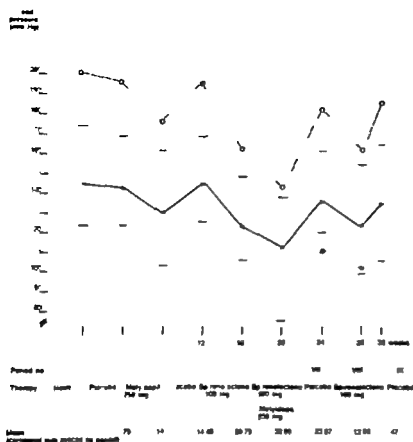


Fig. 1 Mean BP in 28 patients with essential hypertension during the different treatment periods

RESULTS

Fig. 1 shows the average BP for the 28 patients who completed the investigation before and after the treatment periods. The statistical evaluation of the changes in mean BP is given in Table 1.

The patient group as a whole showed a significant fall in mean BP in all the active treatment periods. The strongest effect was obtained when spironolactone 200 mg/day and methyl dopa 750 mg/day were combined (S_2MD). It should however be observed that this period of combined

treatment was not preceded by a placebo period so that the BP did not start from baseline levels. The second best effect was obtained after spironolactone 200 mg/day (S_2) while there was no significant difference in the BP response between methyl dopa 750 mg/day (MD) and spironolactone 100 mg/day (S_1) (Table 1).

The interindividual variation in the BP response was considerable in all treatment periods but the effect was more uniform with S_2 than with MD . A fall in mean BP exceeding 14% was registered in

Table 1a Means and variations after four different treatments. Absolute (mmHg) and percentage reduction (within parentheses) of mean BP from preceding placebo period

MD =methyl dopa (250 mg \times 3) S_2 =spironolactone (100 mg \times 2) S_1 =spironolactone (100 mg \times 1) S_2MD = S_2 + MD

	MD	S_2	S_2MD	S_1	Mean
\bar{x}	14.14 (9.75)	20.75 (14.41)	32.00 (22.36)	12.57 (9.38)	19.87 (13.98)
S.D.	1.28 (8.27)	8.69 (5.61)	10.04 (6.75)	9.14 (6.93)	10.14 (6.95)

Table 1b Analyses of variance of mean BP reductions induced by four different forms of treatment

	D F	Sum of squares	Mean squares	F	Significance
Treatment	3	6 531.46	2 183.82	24.70	$p < 0.001$
Patients	27	3 913.25	145.68	1.65	$p < 0.05$
Errors of trial	111	7 160.29	88.40		
Total	111	17 645.00	198.96		

Significance tests on 6 individual comparisons. Tolerance factor t_{∞} , confidence = 90% conf.int. = $t.S./\sqrt{2/28} = 5.53$
 $MD-S_1 = -6.61$ $MD-S_2MD = -17.86$ $MD-S_3 = +1.57$ $S_1-S_2MD = -11.25$ $S_1-S_3 = +8.18$ $S_2MD-S_3 = +19.43$. All the comparisons except $MD-S_3$ are significant, i.e. the differences are greater than the related confidence interval 5.53

32% of the patients after MD in 50% after S_1 in 89% after S_2MD and in 29% after S_3

In the low PRA group after S_1 the mean BP was reduced by an average of 22.6 mmHg (Table II) compared with 19.9 mmHg in the normal PRA group (Table III) the corresponding reductions after S_2MD were 34.3 and 30.8 mmHg. These differences between PRA groups in the reduction of mean BP were not statistically significant. Neither were any such differences found after MD.

The serum concentrations of potassium, urea, cholesterol and uric acid were determined in most patients before and after two months on S_1 , the last month combined with MD (Table IV). In 26 patients the serum potassium concentration increased significantly ($p < 0.01$) from mean 4.0 to 4.5 mEq/l. In two patients more than 5.0 mEq/l were found at the end of the treatment period with subsequent normaliza-

tion after withdrawal of spironolactone. In 17 patients the mean urea concentration increased significantly ($p < 0.05$) from mean 38 to 49 mg/100 ml in three of these cases levels of 64–79 mg/100 ml were found at the end of the treatment period. The slight decrease in cholesterol and increase in uric acid were not significantly different from the pre-treatment levels.

Side-effects

The most common side-effects were fatigue and dizziness in four patients while on MD in three on S_1 and in six on S_2MD . After treatment with MD three patients developed nausea as did three after S_1 and three after S_2MD . In four of the patients the treatment was withdrawn on account of these side effects, in one after S_1 , in the other three after S_2MD .

Table II Change in mean BP (mmHg) after MD, S_1 and S_2MD in 9 low renin hypertension patients

B before A after furosemide (80 mg). Other abbreviations as in Table I

Pat. no.	Age (y)	Sex	PRA (ng ang. I/ml/h)		BP during placebo period	Change in mean BP after		
			B	A		MD	S_1	S_2MD
1	45	♀	<0.1	0.2	170/115	-14	-27	-34
2	61	♀	0.2	0.2	165/105	-24	-18	-47
3	53	♂	<0.1	0.2	165/105	-13	-17	-39
4	71	♂	<0.1	<0.1	213/120	-13	-29	-38
5	63	♂	<0.1	<0.1	198/117	-9	-26	-35
6	70	♂	<0.1	0.2	220/120	-47	-22	-38
7	88	♀	0.1	<0.1	203/113	+7	-25	-24
8	57	♀	<0.1	<0.1	40/130	-7	-26	-28
9	57	♂	<0.1	<0.1	198/98	-9	-13	-26
Average 58.8 ± 8.1			Low PRA		197/114	-14.3 ± 4.90	-22.6 ± 1.80	-34.3 ± 2.43
Significance						$p < 0.05$	$p < 0.01$	$p < 0.01$

Plasma aldosterone levels were all normal, ranging from 16 to 63 pg/ml

Table III Change in mean BP (mmHg) after MD S_2 and S_2 MD in 19 normal renal hypertension patients. Abbreviations as in Table II

Pat. no.	Age (y)	Sex	PRA (ng ang. I/ml/h)		BP during placebo period	Change in mean BP after		
			B	A		MD	S_2	S_2 MD
1	63	♀	0.3	1.5	220/120	-34	-29	-39
2	44	♂	1.3	1.3	180/130	-10	-19	-19
3	58	♂	0.4	1.2	210/118	-27	-26	-35
4	57	♀	0.4	0.8	180/105	-18	-23	-27
5	50	♂	0.3	0.2	172/117	-23	-26	-12
6	51	♂	0.3	0.2	178/128	+ 6	-18	-45
7	67	♂	0.5	0.6	190/110	- 5	-20	-22
8	111	♂	0.9	0.7	207/118	-28	-10	-19
9	72	♀	0.3	0.3	212/110	-23	- 1	-36
10	48	♀	0.8	1.4	200/110	+ 1	-10	-34
11	66	♂	0.2	1.8	195/127	-16	-43	-39
12	63	♂	0.3	0.2	163/102	0	-12	-24
13	64	♂	0.8	0.6	183/112	-15	-24	-31
14	56	♀	0.2	0.5	215/135	-24	-12	-41
15	33	♂	0.3	0.2	163/103	0	-11	-19
16	52	♂	0.4	0.5	232/132	-24	-20	-30
17	53	♀	0.4	1.4	237/120	-14	-28	-33
18	60	♀	0.4	1.0	208/115	-12	-33	-58
19	50	♂	<0.1	0.3	167/107	- 2	-13	-23
Average 56.2			Normal PRA		195/117	-14.1	-19.9	-30.8
S.E.M.						± 2.65	± 2.27	± 2.54
Significance						$p < 0.01$	$p < 0.01$	$p < 0.01$

DISCUSSION

In most of the 28 patients who completed the trial the hypotensive response observed with S_2 was more pronounced than with MD while there was no significant difference between MD and S_2 . The BP fall was greatest, however, when S_2 and MD were together (S_2 MD). Average falls in mean BP of 14% after S_2 and of 22.4% after S_2 MD indicate that these regimens are alternatives in the medical treatment of essential hypertension.

Recent investigations have shown that patients with essential hypertension form a heterogeneous group in terms of PRA levels, low PRA being found in about 15–30% of all patients with this clinical diagnosis (5). Although increased production of aldosterone or other mineralocorticoids such as corticosterone, deoxycorticosterone or 18-hydroxy deoxycorticosterone has been found in some patients in the low PRA group, the great majority have normal levels of these steroids (5, 11). In some

Table IV Serum concentrations of potassium, urea, cholesterol and uric acid before and after S_2 for 2 months combined with MD during the last month. Abbreviation as in Table II

Potassium (mEq/l)		Urea (mg/100 ml)		Cholesterol (mg/100 ml)		Uric acid (mg/100 ml)	
B	A	B	A	B	A	B	A
4.0	4.5	38	49	300	282	6.2	7.4
(3.1–4.6)	(3.8–5.4)	(21–56)	(30–79)	(227–400)	(175–386)	(4.0–11.6)	(4.8–11.4)
n=26	26	17	17	18	14	14	14
Significance	$p < 0.01$	$p < 0.05$		N.S.		N.S.	

cases however there may be a relative or inappropriate mineralocorticoidism in relation to the sodium and water balance (9-15) or an increased sensitivity to normal steroid levels.

Several investigations indicate that patients with low PRA hypertension respond favourably to treatment with spironolactone (6-8) as in the case of primary aldosteronism (18).

Thus in a double-blind investigation, Carey et al (6) found a strong effect of spironolactone in all cases but one in the low PRA group whereas no significant effect on BP was found in the normal and high PRA group while other authors have found that spironolactone may reduce high BP in normal PRA hypertension as well (12, 14). Adlin et al (2) found that the hypotensive response to spironolactone was the same as that to hydrochlorothiazide in the low PRA group, indicating that these two aetiological regimens might be equivalent.

In the present investigation the average decrease in mean BP after S_2 in the low PRA group with normal plasma aldosterone levels was equal to that in the normal PRA group. Neither did the hypotensive response to S_2 MD differ significantly between the two groups.

The varying hypotensive response observed with spironolactone in different investigations when related to PRA levels, might be due to several factors such as differences in the doses used, duration of the treatment and different criteria in the selection of patients. The method for determining PRA and the interpretation of these data vary considerably indicating the need for international standardization. In the present and in most other similar investigations the criteria for low and normal PRA have been chosen arbitrarily.

In essential hypertension a raised sodium and water content in the vascular wall might be of pathogenic importance by increasing the peripheral resistance (14-25) or the sensitivity to catecholamines (7-20). Several studies indicate that diuretics in long-term treatment may reduce the sodium and water content in the vascular wall and thereby decrease the peripheral vascular resistance (16-4). Since the diuretic response to spironolactone is comparable to that of hydrochlorothiazide (17) the former drug might be a useful alternative alone or in combination with thiazide for counteracting side-effects such as hypokalaemia or to restore body calcium deficiency (10).

In 6 patients in the normal PRA group the

PRA rose 0.6-1.6 ng ang./ml/h two hours after furosemide orally whereas a minor or no increase in PRA occurred in the remainder of this group. In the former patients the average decrease in mean BP after S_2 MD (39.7 mmHg) was significantly greater ($p < 0.01$) than in the other patients in this group (26.8 mmHg). MD is known to decrease PRA levels whereas thiazides as well as spironolactone increase them (1-17, 18). Although the number of patients is too small to allow any conclusions this finding could indicate that combined treatment with methyldopa and spironolactone is more useful in hypertensive patients whose PRA responds to furosemide.

It is well known that diuretics of the thiazide group can induce serious metabolic side-effects, such as reduced glucose tolerance and increased serum uric acid levels when used for long periods. Whether these drugs are liable to influence the serum lipid pattern and contribute to the development of atherosclerosis is a matter of discussion (3-13). Such metabolic side-effects have not been reported during treatment with spironolactone in the doses used in this investigation. A significant increase in serum urea was, however, observed in our patients after spironolactone indicating that kidney function might have been impaired. More serious side-effects such as gynaecomastia, impotence and menstrual disorders were not observed in the present investigation but suggest, when present, that spironolactone may interfere with basic endocrine regulations. It should be observed that a direct inhibitory effect of spironolactone on the adrenal glands has recently been demonstrated (23).

In our low PRA group two of the patients suffered from coronary heart disease and one of them developed cerebral stroke with hemiplegia after this study had been completed whereas similar vascular complications were not observed in the normal PRA group. Although the number of patients is small and the observation time short, our data do not support the concept of Brunner et al (5) that low PRA affords protection against serious vascular complications.

We conclude that diuretics of the thiazide type as well as spironolactone are useful and generally indispensable alternatives as basic therapy in hypertensive disease. More needs to be known about the effects and side-effects in the long-term treatment of subjectively healthy people with clinically diagnosed hypertension.

REFERENCES

1. Acchardo, S., Dustan, H. P. & Tarazi, R. C. Similar effects of hydrochlorothiazide and spironolactone on plasma renin activity in essential hypertension. *Cleveland Clin. Quart.* 39 (4): 153 1972
2. Adl, E., V. Marks, A. D. & Chasonick, H. J. Spironolactone and hydrochlorothiazide in essential hypertension. *Arch. Intern. Med.* 130: 835 1972
3. Bergström, J., Holmsten, E. & Solhjem, S. B. The effect of mefruside on plasma and muscle electrolyte and blood pressure in normal subjects and in patients with essential hypertension. *Acta med. scand.* 194: 427 1973
4. Brown, J. J., Ferri, J. B., Fraser, R., Lever, A. F., Love, D. R., Robertson, J. I. S. & Wilson, A. A review of spironolactone therapy in patients with hypertension, aldosterone excess and low plasma renin. Increased deoxycorticosterone in hypertension—another variant of the mineralocorticoid excess syndrome. In: *Hypertension '72* pp. 313–319. Springer Verlag, Berlin Heidelberg and New York 1972.
5. Brunner, H. R., Larrigh, J. H., Baer, L., Newton, M. A., Goodwin, F. T., Krafoff, L. R., Bard, R. H. & Bühler, F. R. Essential hypertension. Renin and potassium, heart attack and stroke. *New Engl. J. Med.* 286: 441 1972
6. Carey, R. M., Douglas, J. O. & Liddle, G. W. Spironolactone in patient with essential hypertension and suppressed plasma renin activity. *Clin. Res.* 10: 72, 1972
7. Coe, J. W., Cohen, E. L., Lucas, C. P., McDonald, W. J., Mayor, G. H., Bloogh, W. M., J. Everslund, W. C., Beckstein, J. J. & Lapides, J. Primary reninism. Hypertension, hypernatremia and secondary aldosteronism due to renin-producing juxtaglomerular cell tumors. *Arch. Intern. Med.* 130: 682 1972.
8. Craze, M. G. & Harris, J. J. Effect of spironolactone in hypertensive patients. *Amer. J. med. Sci.* 260: 311 1970
9. Davies, D. L., Schalekamp, M. A., Breevers, D. O., Brown, J. J., Briggs, J. D., Lever, A. F., Medina, A. M., Morton, J. J., Robertson, J. I. S. & Tree, M. Abnormal relation between exchangeable sodium and renin-angiotensin system in malignant hypertension and in hypertension with chronic renal failure. *Lancet* 1: 683 1973
10. Edmond, C. J. & Jan, B. Total body-potassium in hypertensive patient during prolonged diuretic therapy. *Lancet* 2: 8 1972.
11. Fishman, L. M., Kuchel, O., Liddle, G. W., Michels, A. M., Gordon, R. D. & Check, W. T. Incidence of primary aldosteronism. Uncomplicated essential hypertension. *J. A. M. A.* 205: 497 1968.
12. Fröhlich, E. D. Summary of discussions. In: *Hypertension '77* pp. 333–336. Springer Verlag, Berlin, Heidelberg and New York 1972.
13. Heyden, S. Epidemiology I. Atherosclerosis pp. 303–313. Elsevier, Amsterdam, London and New York 1969
14. Jose, A., Crout, J. R. & Kaplan, N. H. Suppressed renin activity in essential hypertension. *Ann. Intern. Med.* 72: 9 1970
15. Loeferer, J. A., Beckerhoff, R., Dowdy, A. J. & Wiklison, R. Incomplete suppression of aldosterone secretion and plasma concentration in hypertensive patients on high sodium intake. In: *Hypertension '72* pp. 286–292. Springer Verlag, Berlin Heidelberg and New York 1972.
16. Lund-Johansen, P. Hemodynamic changes in long-term diuretic therapy of essential hypertension. *Acta med. scand.* 187: 509 1970
17. Spark, R. F., O'Hare, C. M. & Regan, R. M. Low renin hypertension. Restoration of normotension and renin responsiveness. *Arch. Intern. Med.* 133: 285 1974
18. Spark, R. F. & Melby, J. C. Aldosteronism in hypertension: the spironolactone response test. *Ann. Intern. Med.* 69: 685 1968
19. — Hypertension and low plasma renin activity: presumptive evidence for mineralocorticoid excess. *Ann. Intern. Med.* 75: 831 1971
20. Støerpel, K. Det farmakologiske grunnlag for script med anti-hypertonika. In: *Diuretika og hypertensjons-symposium* (ed. Bayer Kjønn A.S.) pp. 23–32. Nielsen Oslo 1971
21. Sundsfjord, J. A. Radiolimmunological determination of plasma renin activity during the menstrual cycle and during acute progesterone administration. *Acta endocr. (Kbh.)* 67: 174 1971
22. — Variations in plasma aldosterone and plasma renin activity throughout the menstrual cycle with special reference to the pre-ovulatory period. *Acta endocr. (Kbh.)* 73: 499 1973
23. Sundsfjord, J. A., Marton, P., Jørgensen, H. & Aakvaag, A. Reduced aldosterone secretion during spironolactone treatment in primary aldosteronism. Report of case. *J. clin. endocr.* 1 press 1975
24. Tobian, L. Why do thiazide diuretics lower blood pressure in essential hypertension? *Ann. Rev. Pharmacol.* 7: 399 1967
25. Woods, J. W., Hill, C. H., Liddle, H. W., Stant, E. G., Michelakakis, A. M. & Brill, A. B. Effects of an adrenal inhibitor in hypertensive patients with suppressed renin. *Arch. Intern. Med.* 123: 366, 1969

PLASMA RENIN ACTIVITY BLOOD PRESSURE AND SODIUM EXCRETION DURING TREATMENT WITH CLONIDINE

F Fyhrquist, A Kurppa and M Huuskonen

*From The Minerva Institute for Medical Research and The Lanttahoori
Research Center Helsinki, Finland*

Abstract Clonidine 225 µg a day has been given orally for 3 months to 15 patients with essential hypertension. Mean BP was reduced from 159/107 to 143/87 mmHg. The antihypertensive effect of the drug was dissociated from changes in plasma renin activity (PRA) during clonidine treatment. PRA levels decreased initially in 6 patients with the highest PRA values before treatment, but then increased again. In 6 patients with low or pretreatment PRA levels, PRA rose continuously. Opposite patterns of 24-hour urinary sodium excretion were observed in these arbitrary subgroups. The antihypertensive effect of clonidine in essential hypertension appears to be independent of changes in PRA.

Recent observations that clonidine an antihypertensive drug, decreases plasma renin activity (PRA) when administered i.v. (4, 9) or orally to normo- and hypertensive people (2, 7, 9) have prompted suggestions that suppression of renin secretion may contribute in the antihypertensive effect of clonidine (2, 9). The mechanisms by which clonidine exerts its antihypertensive and renin suppressive effects (9) are incompletely understood. There is evidence for a suppressive effect on central sympathetic vasomotor mechanisms and peripheral effects as well (4, 8).

Previous work on PRA during clonidine therapy has been largely concerned with acute and short time studies (2, 4, 9). Chronic treatment with clonidine reportedly causes suppression of PRA levels in hypertensive patients (7). In patients with essential hypertension, clonidine reported to cause retention of sodium (7, 9) and suppression of urinary aldosterone excretion (2).

We investigated the effect of low oral dose of clonidine on PRA levels, BP and urinary excretion of sodium and potassium in patients with mild, essential hypertension. The results indicate that

suppression of PRA is not a consistent feature during prolonged antihypertensive therapy with clonidine.

PATIENTS AND METHODS

Fifteen patients, 3 of them female, mean age 38.7 years (range 21-55), were included in the study. Excluded were patients with any of the following: signs of secondary hypertension, heart failure, renal or hepatic disease, metabolic or endocrine diseases, psychiatric disorders, known duration of hypertension above 10 years. All had diastolic BP consistently ≥ 100 mmHg, measured in the supine, sitting and upright postures on several occasions during at least 3 months. Individual clinical data are given in Table 1. Only WHO stage I-II hypertension was included. The examination included X-ray of the chest, ECG, pyelography, ECG estimation of Na, K, chlorides, and creatinine concentrations in serum, and urinary excretion of Na and K (flame photometry).

Study protocol. A detailed information sheet was given to each patient. Eleven patients had no previous antihypertensive treatment. The other 4 were without drugs for at least 2 weeks before starting the study. Clonidine, 75 µg 3 times a day (Catapresan, Boehringer Ingelheim) was given orally for 3 months on non-blind, outpatient basis. No dietary restriction was introduced, and ordinary outpatient antihypertensive treatment was mimicked as closely as possible. Each patient was seen immediately before treatment, after 1 month and after 3 months. Each visit to the clinic included clinical examination, BP measurements, and collection of samples for the estimation of PRA, serum concentration of Na and K, and urinary excretion of Na and K.

During the course of the study the physicians examining patients were not aware of PRA data and the laboratory personnel did not know about the clinical data.

Renin assay. Venous blood for PRA assay was collected into ice-chilled tubes containing disodium-EDTA (15 mM final conc.) after 12 hours of fasting and 2 hours of upright posture (walking) at 10-11 a.m. Plasma, separated by centrifugation at +4°C, was stored at -20°C until assayed. PRA was estimated with radioimmunoassay for

Table I Clinical data of the patients studied

LVH=left ventricular hypertrophy

Patient no	Age (y)	Duration of hypertension (y)	Supine BP 1st visit, without drugs (mmHg)	Serum creatinine ($\mu\text{mol/l}$)	Possible complications of hypertension
1	34	3.5	160/100	91	Subarachnoidal haemorrhage in 1972
2	25	6	160/100	88	None
3	41	0.5	165/105	101	None
4	13	1	160/100	86	None
5	44	0.5	155/100	91	None
6	40	0.5	145/100	82	None
7	55	0.5	150/100	85	Slight signs of LVH
8*	43	0.5	170/110	76	None
9*	55	3.5	180/120	97	Slight signs of LVH
10	40	0.5	170/110	82	None
11	41	0.5	160/110	113	None
12	21	0.5	190/110	84	None
13	48	1	170/115	96	Slight signs of LVH
14	33	0.5	180/120	100	None
15	28	2	160/100	85	None
Mean	38.7	1.4	163 \pm 3.1	90 \pm 2.5	
\pm S.E.M.	\pm 2.6	\pm 0.43	107 \pm 1.9		

Female

angiotensin I by a method modified according to the method of Stockigt et al. (10). Reference values for 23rd normotensive healthy subjects under identical conditions were 1.06 ± 0.13 (S.E.) ng/ml/h. Interassay variation was 11% (S.D.) and intrassay variation 8.2%. Significant correlations were found between data obtained from identical plasma samples with the present radioimmunoassay and PRA bioassay according to Boucher et al. (3) ($r=0.77$).

RESULTS

Plasma renin activity After 1 month of clonidine treatment mean PRA decreased from 0.81 to 0.61 ng/ml/h (not significant) and then increased to 1.00 ng/ml/h ($p<0.05$) after 2 further months (Table II). Two patterns of change in PRA could be distin-

guished namely a decrease of the highest levels followed by an increase after 3 months (Fig. 1) and an increase of PRA in those with pretreatment values below the mean followed by either a further increase or decreases with further months of clonidine therapy.

A study of the 6 patients with the highest pretreatment PRA levels and the 6 with low pretreatment and continuously increasing PRA values (Fig. 1) revealed a contrary pattern for sodium excretion.

Blood pressure BP decreased significantly after one month of clonidine therapy (systolic supine and upright $p<0.001$, diastolic $p<0.001$) but did not decrease significantly with 2 further months of drug treatment (Table II). Two of 15 patients had end

Table II Blood pressure, plasma renin activity and urinary 24-hour excretion of sodium and potassium (mean \pm S.E.)

	BP (mmHg)				PRA (ng/ml/h)	Urinary 24-hour excretion (mEq)	
	Supine Systolic	Supine Diastolic	Upright Systolic	Upright Diastolic		Na	K
Pretreatment	158 \pm 3.6	106 \pm 2.3	159 \pm 3.8	107 \pm 2.6	0.81 \pm 0.22	209 \pm 18	78 \pm 6.3
After 1 month	143 \pm 3.4**	92 \pm 2.2**	143 \pm 2.5	93 \pm 2.5**	0.61 \pm 0.11	185 \pm 13	75 \pm 6.0
After 3 months	145 \pm 3.3	89 \pm 2.0	143 \pm 3.2	87 \pm 1.9	1.00 \pm 0.15	204 \pm 20	75 \pm 5.7

 $p<0.05$ * $p<0.001$

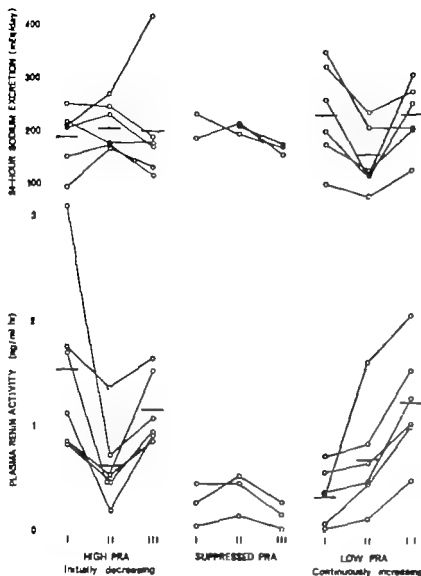


Fig 1 PRA and 24-hour urinary excretion of sodium before treatment (I) after 1 month (II), and after 3 months (III) of clonidine therapy. Mean values are indicated by horizontal lines.

treatment diastolic BP ≥ 100 mmHg and were considered clinical failures. The antihypertensive effect of clonidine was considered good and the side effects observed were few (Table III). Although the best responses of diastolic BP were observed in patients with low pretreatment PRA levels (Fig. 2) no significant correlation was found between diastolic BP decrease and either pretreatment ($N=15$ $r=0.37$) or end-treatment PRA values ($N=15$ $r=0.14$).

Excretion of sodium and potassium The mean pretreatment 24-hour sodium excretion decreased

after 1 month of clonidine therapy (not significant Table II) and returned to pretreatment levels after 3 months of drug treatment. In 5 of the 6 patients with the highest pretreatment PRA levels (Fig. 1), an increase in sodium excretion after 1 month (not significant) was followed by decreased 24-hour sodium excretion. In the 6 patients with low PRA levels before treatment and continuously increasing PRA during clonidine therapy a significant decrease ($p<0.05$) in sodium excretion was followed by an increase ($p<0.05$) back to pretreatment levels.

Table III Antihypertensive effect of clonidine

Duration of treatment (mo)	No. of pts.	Normotensive ^a	Re-positive ^b	Side-effects (no. of pts.)
1	15	8	5	Drowsiness 5 Dry mouth 3 Swollen legs 2 Drowsiness 1
3	15	8	5	

^a BP < 140/90 mmHg (upright).

^b BP < 150/95 mmHg (upright) or BP reduced 20/20 mmHg or more.

Urinary 24-hour excretion of potassium and serum concentrations of sodium and potassium, did not change significantly during the study (Table II).

DISCUSSION

The present study in contrast to previous reports (2, 7, 9) revealed increased PRA levels during clonidine therapy in a considerable proportion of patients with mild essential hypertension. Decreased PRA values were observed in patients with the highest pretreatment PRA levels but even in these patients PRA levels were not significantly suppressed after 2 further months of clonidine therapy. Much of the differences between these results and previous reports may depend on the low dose of clonidine (225 µg a day) used by us. Moreover previous investigations on the effect of nifedipine on PRA levels have been largely con-

cerned with acute (4, 9) or short-time (2, 9) effects, while we studied the effect of clonidine during 3 months. Previous reports (4, 7, 9) on PRA during clonidine administration have been based upon the bioassay method of Boucher et al (3) or radioimmunoassay of angiotensin I (2). Data obtained by our immunoassay method (10) correlated significantly with results using bioassay according to Boucher et al. Therefore it appears unlikely that differences in results could be explained by different renin assay techniques.

Baer et al (2) reported suppression of PRA in 22 hypertensive patients on a constant metabolic regimen and treated with 300 µg clonidine a day for 5 days. They also observed sodium retention and suppression of urinary aldosterone excretion. They suggested that clonidine may be useful for patients with hyperreninemia and aldosteronism. Our data do not favour such a conclusion. Hökfelt et al (7) reported suppression of PRA levels in various types of hypertension with clonidine doses ranging from 300 to 900 µg daily for 6–30 days. However clonidine did not inhibit an increase in PRA in response to sodium restriction in one of their patients.

Clonidine reportedly causes a decrease in renal vascular resistance in patients with essential hypertension during orthostatic stimulation with 45° tilting (9). We measured PRA after stimulation in upright posture. The increased levels of PRA during treatment with clonidine reported here may be due to decreased renal vascular resistance. Other sympathetic inhibitors (reserpine, trimetaphan) reported to increase PRA in dogs with experimental hypertension (1) may cause release of renin by a similar mechanism.

During dietary sodium restriction (30 mEq Na daily) a powerful stimulus for renin release clonidine 100 µg 4 times a day administered orally for 5 days to patients with essential hypertension caused suppression of PRA in both the supine and in the 45° tilted posture (9). This may be explained in part by initial retention of sodium and fluid caused by clonidine (2, 9) in addition to inhibition of sympathetic nervous mechanisms (8). In agreement with such an explanation we observed the most impressive reductions of PRA in the patients with ankle edema and weight gain. During sodium repletion, however, when PRA levels are generally lower the inhibitory effect of clonidine on renal reflex vasoconstriction in response to orthostatic stimulation (9) may override the inhibitory sympathetic

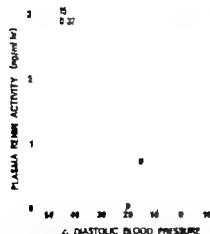


Fig. 2 PRA before treatment compared to change of diastolic BP in upright posture. Linear regression analysis not significant.

nervous mechanisms (8). The net effect would be an increase in PRA during upright posture as observed by us in low renin hypertensive patients.

The antihypertensive effect of the low dose of clonidine (225 µg daily) used in this study appeared better than that reported by Omsal et al. (9) using 400–900 µg a day. The lower dose of clonidine may explain the lower frequency of side-effects observed by us. Antihypertensive responses similar to those reported here were observed by Baer et al. (7) in patients receiving 300 µg clonidine daily for 5 days.

The antihypertensive effect of clonidine was not significantly correlated to pretreatment PRA levels and was dissociated from changes in PRA levels during therapy. This was not altered when our patients were divided into subgroups according to 4-hour urinary excretion of sodium as described by Brunner et al. (5). It would appear that clonidine may be used as an antihypertensive agent even in mild, essential hypertension, regardless of the PRA levels.

The possibility remains that in clinical states with inappropriate hypersecretion of renin, e.g. renovascular hypertension, suppression of renin release may be beneficial. However this may require higher doses as used by Hökfelt et al. (7). Moreover the hypertensive and vasoconstricting effect of exogenous angiotensin and catecholamines in rats is blunted by clonidine (6). Thus clonidine may in fact stimulate renin release while simultaneously counteracting the pressor activity of angiotensin generated by renin.

The opposite patterns of sodium excretion observed in patients with "high" and low continuously increasing PRA levels (Fig. 1) seem to indicate that clonidine causes different changes in sodium excretion and perhaps ingestion in these two arbitrary subgroups of essential hypertension. The tendency towards stabilization of sodium excretion after 3 months of clonidine therapy suggests that the effect on sodium balance is transient fol-

lowed by adaptive adjustments which do not abolish the antihypertensive effect of the drug.

ACKNOWLEDGEMENTS

This study was supported by grants from the Sigrid J. Åelgers Foundation and the Finnish Medical Society.

REFERENCES

- 1 Ayers, C. R., Harris, R. H. J. & Lefer, L. G. Control of renin release in experimental hypertension. *Circulat. Res. Suppl.* 1: 103 1969.
- 2 Baer, L., Brunner, H. R., Bard, R. & Laragh, J. Suppression of renin and aldosterone by clonidine. *Ann. Intern. Med.* 74: 830, 1971.
- 3 Boucher, R., Veyrat, R., De Champlain, J. & Genest, J. New procedures for measurement of human plasma angiotensin and renin activity levels. *Canad. med. Ass. J.* 90: 194 1964.
- 4 Brod, J., Horbach, L., Just, H., Rosenthal, J. & Nicolescu, R. Acute effect of clonidine on central and peripheral haemodynamics and plasma renin activity. *Europ. J. Clin. Pharmacol.* 4: 107 1972.
- 5 Brunner, H. R., Laragh, J. H., Baer, L., Newton, M. A., Goodwin, F. T., Krakoff, L. R., Bard, R. H. & Bühler, F. R. Essential hypertension. Renin and aldosterone, heart attack and stroke. *New Engl. J. Med.* 286: 441 1972.
- 6 Forstner, K., Lindner, A. & Selzer, H. Die Testung von Antihypertensiva mit protrahierter Wirkung. *Wien klin. Wochschr.* 80: 183 1968.
- 7 Hökfelt, B., Hedelund, H. & Dyrnäs, J. P. Studies on catecholamines, renin and aldosterone following Campress® (1-(2,6-dichloro-4-phenylamino)-imidazole hydrochloride) in hypertensive patients. *Europ. J. Pharmacol.* 10: 389 1970.
- 8 Koblinger, W. Pharmacological basis of the cardiovascular actions of clonidine. In: *Hypertension: Mechanisms and Management* (ed. G. Owens, K. E. Kim & J. H. Moyer), p. 369. Croom & Stratton, New York and London 1973.
- 9 Omsal, O., Schwartz, A., Kim, K. E., Paz, Martinez, V. & Swartz, C. Antihypertensive effect of clonidine. *Circulat. Res. Suppl.* 2: 11 1971.
- 10 Stockigt, J. R., Collins, R. D. & Biglieri, E. G. Determination of plasma renin concentration by angiotensin I immunoassay. *Circulat. Res. Suppl.* 2: 175 1971.

GLUCOSE-INSULIN TREATMENT OF LACTIC ACIDOSIS IN PHENFORMIN TREATED DIABETICS

Ole Kristensen, Hans Harrestrup Andersen and Jørgen Borup Jensen

From Medical Departments M and C, Odense H. Spital, Odense, Denmark

Abstract. Four cases of lactic acidosis in phenformin-treated diabetics are presented. Blood lactate before treatment was 5.7, 9.4, 10.7 and 17.8 mEq/l, respectively. Treatment with glucose, insulin and bicarbonate resulted in correction of acidosis and hypertactaemia. This therapy is recommended in phenformin-induced lactic acidosis.

Lactic acidosis in phenformin-treated diabetics has been described since 1959 (19). This type of lactic acidosis is a serious condition, characterized biochemically by accumulation of lactic acid causing metabolic acidosis, and clinically by anorexia, vomiting, and impairment of consciousness varying from lethargy to coma. Various therapeutic principles have been employed: intensive infusion of bicarbonate (1-13), peritoneal dialysis (15), and hemodialysis (17) but mortality has remained high, approximately 50% (5).

Since Johnson and Waterhouse in 1968 (9) presented a case of phenformin-induced lactic acidosis in which glucose-insulin treatment had a remarkable effect, a total of 7 patients surviving after this therapy has been reported (11, 13, 16, 18). This is a report of 4 additional cases occurring in May-Oct. 1974, all successfully treated with glucose, insulin and bicarbonate.

CASE REPORTS

Case 1

A 59-year-old woman who had had mild diabetes for 1 year. One year before admission, treatment of her diabetes was changed from carbamamide to phenformin, 100 mg, and glibenclamide, 5 mg daily. During the last weeks before admission she had lost her appetite. On the day before admission she had suffered from abdominal pain and vomiting. On admission she appeared pale and drowsy. Pulse rate was 104/min, BP 145/85 mmHg, rectal

temperature 36°C. Blood chemistry showed an increased "anion gap" ($(\text{Na} + \text{K}) - (\text{Cl} + \text{HCO}_3^-) > 20$ mEq/l), acidosis, and hypoglycaemia (Table I) indicating lactic acidosis. Treatment consisted of injection of 50 ml 50% glucose + 16 IU soluble insulin, repeated after 3 hours. In addition she received 83 mEq bicarbonate and 1300 ml 5% glucose. Twelve hours after initiation of this therapy her clinical condition had improved and drowsiness had disappeared. Treatment of her diabetes was changed to insulin NPH and she was discharged in good health.

Case 2

A 65-year-old woman with diabetes of 10 years duration, treated with phenformin, 50 mg daily. Five years prior to admission arterial hypertension was diagnosed and treatment with methylglucamine was started. One month before admission she began to lose weight owing to anorexia and frequent vomiting. On the last day before admission she had been confused and had according to her relatives taken overdoses of phenformin and methylglucamine. On admission she was pale, somnolent, confused, and had Kussmaul respirations. BP was 140/80 mmHg decreasing to 100/60. Laboratory data showed metabolic acidosis, hypertactaemia (Table I), and Hb 8.9 g/100 ml. The condition was treated with 50 ml 50% glucose + 10 IU soluble insulin injected i.v. twice, with an interval of 4 hours. In addition 240 mEq bicarbonate and 500 ml blood were given. After 5 hours, acidosis had been corrected but blood lactate was still 8.6 mEq/l (Fig. 1). During the following 8 hours she received 2000 ml 5% glucose + 10 IU soluble insulin. In the same period her clinical condition improved and she became rational. At discharge a satisfactory regulation of her diabetes had been achieved through diet.

Case 3

A 65-year-old woman with diabetes of 10 years duration, treated with phenformin, 100 mg daily, was admitted for treatment of arterial hypertension and epistaxis. On admission moderate arteriosclerotic heart disease she received digoxin and furosemide. After admission medications were continued in unaltered doses. The epistaxis had been stopped by an anterior packing which was removed after 6 days. Two days later she lost appetite and began to vomit.

Table 1 Laboratory data before treatment with glucose, insulin and bicarbonate

	Case no			
	1	2	3	4
Blood lactate (mM/l)	9.4	10.7	5.7	17.8
Plasma glucose (mg/100 ml)	46	209	161	161
Plasma bicarbonate (mEq/l)	11	12	16	5
Plasma chloride (mEq/l)	102	-	88	-
Plasma sodium (mEq/l)	142	136	131	145
Plasma potassium (mEq/l)	5.8	4.0	4.6	5.3
Plasma creatinine (μ M/l)	155	305	265	190
Blood pH	7.17	7.30	7.29	6.79
Blood pCO ₂ (mmHg)	77	18	27	19
Blood O ₂ -sat. (%)	92	97	94	87
Blood base-excess (mEq/l)	-17.5	-15.7	-12.3	-18.7
Urine glucose	0	0	0	+
Urine ketone	+	+	0	++
Urine pH	6	5	-	-

She was pale and hyperventilating. An increased anion gap and a metabolic acidosis suggested lactic acidosis, verified by determination of blood lactate (Table 1). She was treated with 40 ml 50% glucose+16 IU soluble insulin i.v. repeated after 4 hours, 166 mEq bicarbonate and during the following days with insulin NPH and continuous infusion of 5% glucose. After an initial rise, blood lactate decreased, and normal values were reached after 4 hours (Fig. 1). The course thereafter was uneventful. left hospital with a diabetes regimen consisting of diet

Case 4

A 74-year-old diabetic woman, treated with insulin for 18 years, was admitted because of vertigo. After admission regulation of her diabetes was found unsatisfactory and treatment with diet and phenformin was instituted. After 10 days, the dose of phenformin was increased from 50 to 75 mg daily and 8 IU insulin NPH was added. Preceded by anorexia and drowsiness, the patient was found one morning 15 days later deeply comatose. Her respirations were rapid and deep and the pulse was 120/min, BP 180/90 mmHg and temperature 37.1°C decreasing to 35.1°C. An i.v. drip containing Ringer lactate was started. Owing to the finding of normoglycaemia and a pronounced metabolic acidosis (Table 1) lactic acidosis was suspected. Ringer lactate was discontinued after 5 hours, and blood samples showed blood lactate 18.7 mM/l. At this time she had received 500 ml Ringer lactate (14 mM lactate) a dose insufficient to explain the marked elevation of blood lactate. Treatment consisted of i.v. injection of 50 ml 50% glucose+1 IU soluble insulin repeated after 5 hours, 750 mEq bicarbo-

nate and 1000 ml 5% glucose+24 IU soluble insulin given i.v. over 4 hours. Twenty-four hours after institution of this therapy arterial pH was 7.46 and the patient was awake. During the remainder of the hospitalization her diabetes was well controlled with diet and insulin.

DISCUSSION

Biochemical changes in phenformin-induced lactic acidosis consist of an accumulation of lactic acid reflected in an increased anion gap leading to metabolic acidosis. Ketosis is absent or moderate hypoglycaemia is frequently present (1). Lactate is formed from pyruvate and is an end product in anaerobic glycolysis. Lactate is eliminated only after reconversion to pyruvate which in turn is partly oxidized in Krebs cycle partly utilized in gluconeogenesis (11).

The exact mode of action of phenformin is unknown. Based on animal *in vitro* investigations and on some human experiments several mechanisms have been suggested. However their relative contributions in the diabetic human organism are not clear (5). These mechanisms are: increased glycolysis (7, 10), possibly due to an inhibition of mitochondrial respiration (3, 7) reduced gluconeogenesis (4, 7, 14) increased muscular glucose uptake (2, 7) and decreased intestinal glucose absorption (6, 12). These primary actions of phenformin tend to induce a secondary reduction of glucose level and under special circumstances a marked increase in lactate level resulting in acidosis.

Temporarily or persistently impaired renal function which was found in all four cases is consid-

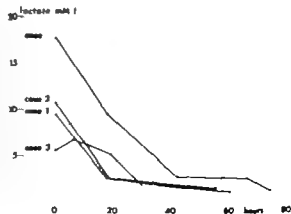


Fig. 1 Blood lactate during treatment with glucose, insulin and bicarbonate

ered an important pathogenetic factor probably due to reduced elimination of phenformin (5-13). Treatment of lactic acidosis with bicarbonate or dialysis has mainly been directed towards a correction of this acidosis. It is doubtful however whether the primary disturbances in glycolysis and gluconeogenesis are affected. The action of glucose-insulin in lactic acidosis is unknown. Johnson and Waterhouse (9) suggested that a block in pyruvate utilization was relieved. However it seems important to attempt to maintain a normal intracellular glucose metabolism through administration of glucose and insulin.

Considering the high mortality in phenformin-induced lactic acidosis and the favourable experience with a therapy consisting of glucose, insulin and bicarbonate this treatment as outlined in the present study is recommended.

REFERENCES

- 1 Bengtsson, K., Karlberg, B. & Lindgren, S. *Acta med. scand.* 191-203 1977.
- 2 Britterfield W. J. H. & Whiclow M. J. *Lancet* 2: 785 1968.
- 3 Davidoff F. *J. Biol. Chem.* 246: 4017 1971.
- 4 Haackel, R. & Haackel, H. *Diabetologia* 8: 118, 1972.
- 5 Hermann, L. S. *Dan. med. Bull.* 20: 65 1973.
- 6 Hollibaugh S. L., Rao, M. B. & Kruger F. A. *Diabetes* 19: 45 1970.
- 7 Jangard M. D. Pereira, J. N. & Finson, R. *Diabetes* 17: 96, 1968.
- 8 Jensen H. *Arcand. Dan. med. Bull.* 20: 16, 1973.
- 9 Johnson, H. K. & Waterhouse C. *Arch. intern. Med.* 122: 367 1968.
- 10 Kreisberg R. A. *Diabetes* 17: 431 1968.
- 11 — *New Engl. J. Med.* 287: 132 1973.
- 12 Kruger F. A., Altschuld R. A., Hollibaugh, S. L. & Jewett, B. *Diabetes* 19: 50 1970.
- 13 Lindgren, S. *Läkartidningen* 66: 3705 1969.
- 14 Lyngsoe J. & Topp-Jensen, J. *Brit. med. J.* 2: 224 1969.
- 15 Madson K., Høier & Andersen, M. *Ugeskr. Læg.* 134: 1280 1972.
- 16 Mestman, J. H., Pocock D. S. & Krehmer A. *Calif. Med.* 111: 181 1969.
- 17 Selroos, O., Pasternack, A. & Kobbäck, B. *Nord. Med.* 80: 1658 1968.
- 18 Shurita, G. G. & Bewsher P. D. *Brit. med. J.* 3: 906, 1970.
- 19 Walker R. S. & Linton, A. L. *Brit. med. J.* 2: 1005 1959.

DIPPING PROCEDURE FOR BLOOD GLUCOSE DETERMINATION WITH DEXTROSTIX AND THE EYETONE REFLECTANCE METER

Assessment of a Practical Technique

C. Kjøhl

*From Department of Internal Medicine T, Buhlshøj Hospital
University of Copenhagen, Copenhagen, Denmark*

Abstract. A dipping procedure for blood glucose determination with the Dextrostix Eyetone system has been evaluated. The procedure involves the immersion of the Dextrostix reagent area for 1 min in a tube of whole blood followed by wash, blotting and reading as in the regular procedure. Sixty-five blood samples covering wide glucose concentration ranges were estimated for their glucose content in random order both by the dipping procedure and conventional Dextrostix Eyetone procedure. An almost perfect agreement between the two methods was found, the coefficient of correlation being 0.99 and the regression line very close to the ideal line. The presence of

Dextrostix reagent area in the blood was found to bring about glycolysis. Except at high blood glucose levels, this glycolysis, however, was insignificant if the strip was correctly removed after 1 min. The dipping procedure overcomes the main technical problem of conventional procedures: the inconsistency of the drop application on the reagent area. A. it is easy to perform and a reliable alternative to conventional procedures. It is recommendable in all cases where blood samples are available.

In a recent Scandinavian multicentre study blood glucose determination with the new Dextrostix reflectance meter Eyetone, was found to be a quick and very reliable alternative to conventional laboratory methods (4). However, this new alternative still poses a technical problem: using conventional techniques it is often difficult to get the complete reagent surface of the strip covered with blood within sufficiently few seconds to ensure exact timing of the procedure. In addition, the amount of blood applied may influence the result (1, 2).

For this reason an alternate procedure for Dextrostix, in which the strip is immersed in a sample of whole blood obtained from a puncture, has been introduced in our laboratory. This report concerns a comparison of the new method with the current one.

MATERIAL AND METHODS

Samples of capillary blood were taken in heparinized tubes from both non-diabetics and diabetics. Hypoglycemic specimens were also collected from patients submitted to an insulin-hypoglycemia test for other reasons, and hyperglycemic specimens were also prepared by adding concentrated aqueous glucose solution. A total of 65 samples were investigated in triplicate and random order by both of the following procedures:

The regular procedure: according to the operating manual for handling the Dextrostix strip and the Eyetone instrument. Blood was applied to the reagent area of the strip by means of a pipette. The Eyetone instrument was supplied by Dr A. Wagner Ames Co., Scandinavia.

The dipping procedure: 1) The end of the Dextrostix strip opposite the reagent area was bent. 2) The Dextrostix was then inserted into the tube of well mixed blood, the bent end being allowed to hook onto the rim of the tube and thus prevent the strip from falling in. 3) The Dextrostix was left in the blood for exactly 60 sec. 4) Thereafter the strip was removed, washed, blotted and read as in the regular procedure.

The reagent area of the Dextrostix strip is covered with a semipermeable membrane which permits the glycolytic enzymes to escape into the surrounding blood. Consequently the blood glucose concentration in the sample might decrease during the dipping procedure and thus invalidate control readings on the same sample. To evaluate the importance of this induced glycolysis, the blood glucose concentration was determined by means of an AutoAnalyzer (Hexokinase-procedure (5)) in each of three samples containing various amounts of glucose. On each sample (3 ml) sixfold estimations were carried out before and 1, 2, and 3 min after the insertion of Dextrostix strip into the sample.

Calculations

Orthogonal regression analysis was applied to test the correlation between blood glucose determination with the two methods, and analysis of variance was used to estimate their precision. The significance of differences between variances was established by means of the *F* test, and differences between means by Student's *t*-test. *P* values less than 0.05 were considered significant.

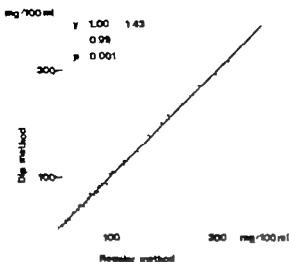


Fig. 1 Correlation between the regular and the dipping procedure for blood glucose determination with the Dextrostix-Eyetone system. Each point is the mean of corresponding triplicate measurements.

RESULTS

Correlation between the two Dextrostix-Eyetone procedures

In Fig. 1 the corresponding mean values of the two methods are plotted against each other. The coefficient of correlation is 0.99 and the regression line $=1.00x+1.43$ very close to the ideal line. This indicates a high degree of correlation between the regular method and the proposed alternative.

Mean and variance

A summary of the precision and variance data is given in Tables I and II respectively. Both the means and the S.D. of the two methods are quite similar. Moreover, the variance of the two methods is not significantly different at any level of blood glucose within the working range of the Eyetone instrument.

Escape of glucose enzymes from the Dextrostix into surrounding blood sample

Immersion of a Dextrostix strip for 3 min in 3 ml blood induced a significant fall in the glucose level of each of three samples investigated (Fig. 2). However, immersion for 1 min significantly suppressed the glucose level only in the sample with the highest glucose content.

Table I Precision of the regular and the dipping method

	Regular method	Dipping method
Mean	130.0	131.3
S.D.	4.8	4.7
Coefficient of variation	3.7	3.6

Table II Variance of the regular and the dipping method at different levels of blood glucose

Range (mg/100 ml)	No. of determinations	Variance		Comparison of variances
		Regular method	Dipping method	
10-30	15	2.60	4.60	n.s.
31-100	1	6.17	7.40	n.s.
101-140	7	11.95	5.86	n.s.
141-200	4	29.17	32.83	n.s.
201-250	6	66.33	31.22	n.s.
251-300	5	43.33	66.67	n.s.
301-350	6	68.06	48.61	n.s.
351-400	1	100.00	100.00	n.s.
10-400	65	22.80	22.29	n

n.s. = no significant difference

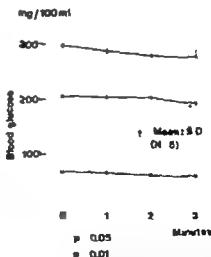


Fig. 2 Influence of immersion of the Dextrostix strip for 1-3 min on the blood glucose level of the sample. Δ = significant suppression of blood glucose concentration.

DISCUSSION

The results indicate almost perfect agreement between the dipping and the conventional Dextrostix-Eyetone procedures. In the latter case the blood was applied with a pipette in order to cover the reagent area completely within a few seconds. This ensures exact timing, which is necessary to obtain precise results (2). In other words, the dipping procedure was tested against the best of the current procedures.

The applicability of the dipping procedure is limited by the need to have a fresh venous blood sample. However, as the Eyetone instrument is as accurate for all practical purposes as the automated apparatuses in general use (4) it may gain access to fields (e.g. hospitals) where blood samples taken for other reasons are often available. In such cases the dipping procedure is a reliable alternative to the current finger-prick or earlobe prick procedures. It is simpler to perform than any of the current procedures including application by a pipette. Furthermore it completely overcomes the inconsistency of the drop application in covering the complete reagent surface of the Dextrostix.

The glycolysis in the sample induced by the immersed Dextrostix strip was insignificant except at high glucose levels provided the strip was in fact

removed after 60 sec. And anyhow this glycolysis is of little practical importance because control readings of blood glucose concentration on the same sample are not indicated as a rule. Another problem is the spontaneous glycolysis in the sample under study which necessitates storage of the sample at 4°C if not analysed immediately (3).

Bearing in mind these small limitations it may be concluded that the dipping procedure for Dextrostix is a reliable alternative to conventional methods and can be recommended whenever whole blood samples are available for other reasons.

REFERENCES

1. Andersen, E. S. & Simonsen, H. E. Blood glucose determinations with Dextrostix reflectometer. *Ugeskr. Læg.* 134: 1332, 1972.
2. Köhl, C. Reliability of blood sugar measurements with Ames reflectometer. *Ugeskr. Læg.* 135: 66, 1973.
3. Scherstén, B. Clinical evaluation of rapid enzyme strip method (Dextrostix) for blood glucose estimation. *Acta med. scand.* 178: 583, 1965.
4. Scherstén, B., Köhl, C., Holbønder, A. & Ekman, R. Blood glucose measurement with Dextrostix and new reflectance meter. *Brit. med. J.* 3: 384, 1974.
5. Widdowson, G. M. & Penton, J. R. Determination of serum or plasma glucose on the AutoAnalyzer II by use of the hexokinase reaction. *Clin. Chem.* 18: 299, 1972.

SUBCUTANEOUS ADMINISTRATION OF SODIUM L THYROXINE TO PATIENTS WITH HYPOTHYROIDISM

J G Ljunggren and B Persson

From the Departments of Endocrinology & Metabolism and Surgery Karolinska sjukhuset
Stockholm S-141 86

Abstract Two patients with primary hypothyroidism are described in whom an euthyroid condition could not be achieved by oral administration of L-thyroxine, L-triiodothyronine or desiccated thyroid. Daily subcutaneous injections of L-thyroxine normalized the thyroxine and triiodothyronine level in the blood and the patients became euthyroid.

Treatment of patients with hypothyroidism is usually no problem since oral administration of L-thyroxine, L-triiodothyronine or desiccated thyroid extract corrects the symptoms and signs of hypothyroidism. Exceptions to this are extremely rare even for a thyroid clinic with a large number of thyroid patients.

This report describes two patients with hypothyroidism in whom the hypometabolic state could not be corrected by oral administration of thyroid hormones. An euthyroid condition was achieved with daily subcutaneous injections of L-thyroxine.

MATERIAL AND METHODS

Subject The first patient was a 30-year-old housewife who developed hypothyroidism after subtotal thyroidectomy for an atypical nodular goiter. She developed typical clinical and laboratory signs of hypothyroidism which could not be corrected by oral administration of thyroid hormones. She was under observation several times in the ward and we have no doubt that she took her pills. The replacement dose was increased but even with 0.4 mg L-thyroxine plus 0.06 mg L-triiodothyronine no improvement was observed in the patient's pronounced clinical and laboratory signs of hypothyroidism.

The second patient was a 72-year-old single woman who for several years had been successfully treated with desiccated thyroid extract for primary hypothyroidism caused

by Hashimoto's thyroiditis. The firm that produced her desiccated thyroid pills had stopped manufacturing them some years previously; the patient had bought a large stock of pills just before production was terminated and she was clinically euthyroid. The laboratory tests were within the normal range while she took her pills and the problem started when her doctor tried other thyroid tablets. She was subsequently referred to our hospital for investigation and the results demonstrated that no other thyroid pills could be used. She experienced severe abdominal discomfort about an hour after taking the new drugs. We tested all available drugs containing L-thyroxine, L-triiodothyronine or desiccated thyroid.

Laboratory tests. The serum concentrations of thyroxine and triiodothyronine were determined by a radioimmunoassay technique (4). The normal range for thyroxine is $6.9 \pm 1.3 \mu\text{g}/100 \text{ ml}$ (mean \pm S.D.) and for triiodothyronine $115 \pm 22 \text{ ng}/100 \text{ ml}$ (mean \pm S.D.). TSH was measured with commercial kit based upon radioimmunoassay technique.

L-thyroxine for parenteral administration was prepared from stock solution containing the sodium salt of L-thyroxine (1.0 mg), sodium derivatives of formaldehyde sulphoxylate (2.0 mg) and sodium salt of acetic acid (4.1 mg), sodium hydroxide (1.8 mg) and sterile water ad 1.0 ml. The final concentration of L-thyroxine was 0.4 mg/ml at pH 10.5.

RESULTS

Parenteral administration of L-thyroxine was initiated when it became obvious that further oral administration of L-thyroxine, L-triiodothyronine or desiccated thyroid extract would have no therapeutic effect. The instruction to the patients regarding the subcutaneous administration was given in the same way as to patients with diabetes mellitus on insulin therapy.

Daily subcutaneous injections of 0.1–0.3 mg L-thyroxine completely reversed the hypothyroid condition. Both patients were clinically euthyroid after two month treatment. The serum levels of

thyroxine was 9.6 and 14.2 $\mu\text{g}/100\text{ ml}$ and of triiodothyronine 108 and 100 $\text{ng}/100\text{ ml}$ in the two patients. TSH was within the normal range. No untoward reactions have as yet been seen since the initiation of therapy.

DISCUSSION

The results demonstrate that an euthyroid condition can be achieved by daily subcutaneous injections of l-thyroxine to hypothyroid patients. The patients can give themselves the injections without any problems.

I.v. administration of thyroid hormones mainly l-triiodothyronine has been described for the treatment of myxoedema coma (1, 3, 5, 6). However, no previous studies seem to have been published concerning the long-term treatment of hypothyroidism with subcutaneous administration of thyroid hormones. Also these patients have been followed up with respect to dose and hormone level in the blood.

The underlying reason for the untoward reaction to oral administration of the thyroid hormones is not fully understood. It is possible that the first patient has a defective absorption of thyroid hormones in the gastrointestinal tract. It is also conceivable that the abdominal discomfort experienced by the second patient is an allergic manifestation which in all probability is not caused by the thyroid hormones as such but possibly by another factor present in the tablets.

This investigation also verifies that thyroxine is converted to triiodothyronine in the body as a normal level of triiodothyronine was found after the administration of thyroxine. The tissue responsible for this conversion is not yet known but this investigation seems to exclude the gastrointestinal tract as a possible site.

The concentration of thyroxine was above the normal range despite a normal triiodothyronine level and an euthyroid condition. This is seen in patients maintained on l-thyroxine and is probably caused by the absence of the triiodothyronine formed by the thyroid gland.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (No. 873-19X/2442 OSA), the Karolinska Institute and the Nordic Insulin Fund.

REFERENCES

1. Forester C. F. Coma in myxoedema. *Arch. Intern. Med.* 111: 734, 1963.
2. Holvey D. N., Goodner C. J., Nicoloff J. P. & Downing J. T. Treatment of myxoedema coma with intravenous thyroxine. *Arch. Intern. Med.* 113: 89, 1964.
3. Ivy H. K. Myxoedema precoma. Complications and therapy. *Mayo Clin. Proc.* 40: 403, 1965.
4. Ljänggren J. G. & Persson B. Determination of T₄ and T₃ in human serum by radioimmunoassay. To be published.
5. Nicoloff J. T. Treatment of hypothyroidism and myxoedema coma. *Mod. Treat.* 6: 463, 1969.

THE SMOKING HABITS OF MEN WITH INTERMITTENT CLAUDICATION

Hans Lithell, Hans Hedstrand and Roland Karlsson

*From the Departments of Geriatrics and Internal Medicine
University Hospital Uppsala, Sweden*

Abstract Smoking habits among 54 male patients with intermittent claudication (IC) and 200 healthy 50-year-old men from the same county have been studied. The prevalence of smokers at the age of 50 was 98% among the IC patients against 46% among the healthy controls. The percentage of heavy smokers and the total tobacco consumption were not significantly different in the two groups. However, the percentage of smokers who began to smoke before the age of 15 was significantly higher in IC patients than in the healthy group, 28% to 7%.

Many studies have shown that the majority of patients suffering from intermittent claudication (IC) are smokers. The frequency in these studies often approaches 100% (3, 6, 9, 10).

When the prevalence of smoking among patients with IC is discussed, reference should be made to the prevalence in the general community. The frequency of smoking among middle-aged men is high in many countries: 70% in the USA and England, 69% in a Swiss population (12), 78% in a Danish study (5) and 70% in a Norwegian study (4). In Sweden the figures are lower: 50% in Uppsala (7), 62% in Malmö (8).

Few of the earlier studies include a control group. The results of a comparison of the smoking habits of men with and without IC from a restricted geographical area are reported here against the background of this regional variation. The results are related to earlier studies.

MATERIAL AND METHODS

Free health examinations have been offered to all men Uppsala born in 1970-74. The main object of the examination was to clarify and treat cardiovascular risk factors.

Requests for reprints to: H. Lithell, Department of Geriatrics, Box 641, S-751 27 Uppsala, Sweden.

Two hundred men born in 1923 were selected at random among these subjects to compare their smoking habits with those of a group of 54 patients with IC.

A total of 170 patients with IC (132 men, 38 women) were investigated at the Geriatric Clinic in Uppsala between May 1971 and June 1973; the majority, however, being referred from other counties or very old. Among these 54 male IC patients born after 1903 from the county of Uppsala were selected for study of their smoking habits. The selection was performed to make the patient and control groups as comparable as possible with regard to place of domicile and age.

A detailed history of smoking habits was obtained at the health control described above. Smoking habits were clarified from the age at which the subjects began to smoke up to the time of examination. Changes in habit were specially examined. The same questionnaire was used in telephone interviews with IC patients. A small group of patients who could not be contacted by telephone were questioned by letter and replies were obtained from 100% of IC patients.

RESULTS

Fig. 1 shows the age distribution (mean 61.0 mean 62.5 years) and number of smokers in the IC group. The number of IC patients who had smoked 5 years before the study is also shown. Forty-one patients (76%) were smokers in 1973 while 52 (96%) had smoked in 1968. Only one patient stated that he had never smoked. This man suffered from diabetes mellitus, hypertension and hyperlipidemia. One patient smoked between the age of 12 and 50 but stopped before 1968. Of the 200 healthy men born in 1923 97 (46%) stated that they smoked at the time of the examination in 1973 while 112 (56%) had smoked 5 years earlier. Sixty-one men (31%) stated that they had never smoked.

Smoking habits between 1968 and 1973 were examined in an effort to illustrate the effect on them of disease. (It was found that in a majority of the

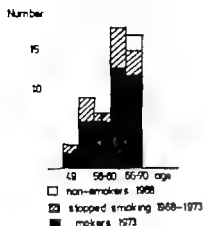


Fig. 1 Age distribution and number of smokers of 54 men with intermittent claudication in 1968 and 1973

patients their disease had begun between these years) Eleven (21%) of the IC patients stopped smoking compared with 20 (18%) controls. A further 17 (23%) of the smoking IC patients and 5 (4%) of the smoking controls reduced their tobacco consumption.

As a large number of IC patients had altered their smoking habits the daily tobacco consumption in 1968 was estimated. As shown in Table I there were no obvious differences between the two groups. Thus there was no overrepresentation of heavy smokers in the IC material.

Total tobacco consumption up to the age of 50 was found to average 140 kg for IC patients and 151 for controls. Table II shows the consumption divided into three arbitrary classes. The percentage of smokers who had consumed more than 250 kg tobacco was almost identical in both groups. A higher proportion of the IC patients had consumed

Table I Tobacco consumption in 1968 of men with intermittent claudication ($n=52$) and of controls ($n=112$)

Relative frequency is given for IC patients to the left of the slanting line and for controls to the right

Pipe tobacco (g/wk)	No. of cigarettes/day	0	1-10	11-20	>20
0		19/23.4	25.0/27.7	7.7/6.3	
<50	21.1/17.0	15.1/7.1	3.8/11.6	—/0.9	
>50	5.8/5.4	1.9/—		—/0.9	

Table II Total consumption of tobacco up to the age of 50 in 53 men with intermittent claudication and in 139 controls

Tobacco consumption (kg)	IC patients		Controls	
	N	%	N	%
<100	19	34	62	45
101-250	30	57	61	44
>250	5	9	16	11

101-250 kg tobacco but this difference is not significant ($\chi^2=2.49$, $p>0.05$).

The age at which IC patients started to smoke averaged 16.8 years whereas for the controls it was 19.1 years. This difference is significant ($p<0.005$). A subdivision into three arbitrary groups is presented in Table III. 28% of IC patients and 7% of the smokers in the control group began to smoke before the age of 15. This difference is also significant ($p<0.005$). Table IV shows the average age of commencing to smoke and illustrates its variation among IC patients divided into 5-year groups according to year of birth. The duration of smoking up to the age of 40 was 32.2 ± 3.4 years (mean \pm S.D.) in the IC patients against 28.5 ± 6.0 years in the controls. This difference is significant ($p<0.001$).

Table III Age at which 53 men with intermittent claudication and 139 controls began to smoke

Age (y)	IC patients		Controls	
	N	%	N	%
<15	15	28	10	7
15-19	25	47	79	57
≥ 20	13	25	50	36

Table IV Age at which 53 men with intermittent claudication began to smoke classified according to year of birth

	Year of birth				
	1903-07	1908-12	1913-17	1918-22	1923-
N	16	18	7	8	4
Range	12-20	14-23	11-25	13-20	17-25
Mean age (y)	15.5	18.2	16.6	15.8	17.8

DISCUSSION

Of the studies listed in the introduction only that by Juergens *et al* (9) has any form of control material. This was a group of patients from the same clinic aged 25-55 who did not suffer from vascular disease. Of them 74% were smokers. We had the opportunity to compare our patients with healthy 50-year-old men, selected from a population study. In view of the possibility of regional variations in the prevalence of smoking, the smoking habits of male IC patients from the county of Uppsala were studied. Only IC patients born after 1903 were selected, in an attempt to reduce the age difference between patients and controls while yielding a suitably-sized patient group. The IC patients were also questioned about their smoking habits at the age of 50 and 98% stated that they had smoked at that age against 46% of the controls. Thus the present study reveals that even when the IC group is recruited from a population with a relatively low prevalence of smokers, it still contains the high proportion of smokers previously reported. Further it should be noted that in 1973 when the IC patients had a mean age of 61 years 76% of them smoked a surprisingly high figure as the prevalence of smoking decreases with age.

Smoking has been discussed as a causative factor for cardiovascular disease especially after Astrup (1) showed the importance of the carbon monoxide concentration in the blood for the development of atheromatosis in the vessel wall. On the other hand when discussing findings such as the present overrepresentation of smokers among IC patients it must be born in mind that other factors (psychosocial, alcohol intake, etc.) may be causative and that smoking just co-varies with these. This study was limited to an examination of the smoking habits alone to see if these differed from those of healthy men.

If smoking is a causative factor for IC one would expect to find an overrepresentation of heavy smokers among IC patients. Juergens *et al* (9) found no difference in numbers of heavy smokers among IC patients and controls. We have investigated tobacco consumption both as total consumption up to 50 years of age and as daily consumption during 1968 and found no difference between our two groups. The year 1968 was chosen as many IC patients changed their smoking habits after the debut of disease between 1968 and 1973.

The lack of correlation between number of heavy

smokers and IC disease may be due to several factors. Wald *et al* (11) have shown that the prevalence of vascular disease is better correlated to carboxyhaemoglobin concentration in blood than to tobacco consumption; this may be due to the fact that individuals with the same tobacco consumption may inhale to different degrees.

Other factors concerning smoking might be of importance. It is noteworthy that the percentage of smokers in our study who began to smoke before the age of 15 is significantly higher among the IC patients than among the controls, 28% to 7%. It has been common only in recent decades for young teenagers to start smoking. Table IV shows however that throughout the age range in the IC group a number of patients started smoking before the age of 15. This low age besides involving more years of exposure to carbon monoxide might also mean that more immature and vulnerable tissues are exposed to carbon monoxide. It is possible that starting smoking at a young age is a factor of importance for the development of arteriosclerotic vascular disease.

The number of patients who ceased smoking during the investigated 5-year period was not greater than the number of those who stopped spontaneously in the control group. All patients in the IC group had been given active anti-smoking advice. Burt *et al* (7) reported that up to 60% of smokers who suffer a heart attack and are actively advised to stop smoking are non-smokers one year afterwards and the same tendency has been found in a local material (to be published). This may of course be due to the fact that a heart attack is often more dramatic and may make the patient more receptive to anti-smoking advice. However the earlier one begins to smoke the more firmly founded might be the smoking habit and if so this may be of importance in that the IC patients seem to have difficulty in giving up smoking.

In this study we have not had the opportunity to investigate the smoking habits of myocardial infarction patients, but this will be done in further studies.

REFERENCES

1. Astrup, P. Some physiological and pathological effects of moderate carbon monoxide exposure. *Brit med J* 4: 447-1972.
2. Burt, A., Illingworth, B., Stans, T. R., Thornley, P., White, P. & Turner, R. Stopping smoking after myocardial infarction. *Lancet* 304: 1974.

- 3 Eastcott, H. H. G., Arterial surgery p. 87 Pitman Medical London 1969
- 4 Ellertsen, E. & Solcheim, P., Rökning och koronar sjukdom Bergenstudien. *Läkartidningen* 67 145 1970
- 5 Hagerup, L. & Larsen, M. Tobaksrygning och respiratoriske symptomer i en dansk population. *Ugeskr. Læg.* 133 1967, 1971
- 6 Haase, H. M. Raucherfragen und Gefasskrankheiten. I: *Angiologie* (ed. M. Ratschow) p. 615 Thieme Stuttgart 1959
- 7 Hedstrand, H. & Waern, H. Ändringar i rökvanor hos män i Uppsala efter 40 års ålder. *Läkartidningen* 70:2761 1973
- 8 Isacson, S. O. Venous occlusion plethysmography in 55-year old men. A population study in Malmö Sweden. *Acta med. scand.*, Suppl. 537 1972.
- 9 Juergens, J. Barker, N. W. & Hines, E. A. Arteriosclerosis obliterans. Review of 520 cases with special reference to pathogenic and prognostic factors. *Circulation* 21 188 1960.
- 10 Räf, L. E., Rökning och claudicatio intermittens. *Läkartidningen* 66:4022 1969
- 11 Wald, N. Howard, S. Smith, P. H. & Kjeldsen, K. Association between atherosclerotic diseases and carboxyhaemoglobin levels in tobacco smokers. *Brit. med. J.* 1 761 1973
- 12 Widmer, L. K. Hartmann, G. Dachosal, F. & Plechl, S.-Ch. : Risikofaktoren und Gefässverschlüsse. *Dtsch. med. Wochr.* 21 1107 1969

STUDIES IN ASYMPTOMATIC PRIMARY HYPERLIPIDAEMIA

II Clinical Findings

Anders U Olsson

From King Gustaf V Research Institute and the Department of Internal Medicine (Lipid Unit), Karolinska Institute at Stockholm, Sweden

ABSTRACT A clinical and laboratory examination of abnormalities not attributable to atherosclerosis has been performed on 183 male and 126 female adult subjects with hyperlipidaemia. The sample was recruited from 20 000 subjects screened at a health control centre who had an initial serum cholesterol and/or triglyceride (TG) concentration above 350 mg/100 ml and 3.50 mmol/l. All were subjectively healthy and had no history of atherosclerotic disease. Known cases of secondary hyperlipidaemia were excluded. Lipoprotein (LP) analysis with preparative ultracentrifugation and electrophoresis was made on all subjects including control groups with "normalised" serum lipids. Typing of hyperlipoproteinaemia (HLP) was performed according to the modified system of Fredrickson et al. Compared to controls, subjects who had elevated very low density LP (VLDL) (types II B, III, IV and V) were obese, while subjects with type II A HLP were shorter. There were more smokers among men with type II B and women with type IV HLP than in the control groups. Arcus corneae was seen in 29% of controls and in higher frequencies in types II A and II B. A positive correlation existed between the frequency of arcus corneae and the mean low density LP cholesterol in the different types. Multiple tendinous xanthomata ($n=11$) were found exclusively in type II A HLP, palmar xanthomata ($n=3$) only in the presence of "floating β -LP" and eruptive xanthomata in one male with type V HLP. The mean ESR was increased in all types of HLP. The mean S-GPT and uric acid concentrations were higher in type IV HLP in both sexes than in the control groups. In men with type IV HLP S-GPT was positively correlated to VLDL TG. The uric acid level was correlated to both the VLDL TG concentration and body weight independently. Of the male subjects with HLP 1/3-1/2 had a diabetic or borderline L glucose tolerance.

Certain clinical features of primary hyperlipoproteinaemia (HLP) are well known. One of them is the high frequency of atherosclerotic disease. Other well known clinical findings are for example xanthomata. There is however no general clinical description of the different kinds of primary HLP. The purpose of this paper is to report clinical findings in a sample of apparently healthy subjects who presented with marked HLP at a health control screening. In parallel studies of the same subjects the occurrence of preclinical signs of atherosclerosis with different locations was assessed (20-31).

METHODS

The sample of subjects with HLP has been described in detail regarding screening procedures, criteria for participation and data on lipoprotein (LP) concentrations (19). In brief it consisted of 314 subjectively healthy men and women exhibiting marked hyperlipidaemia, i.e. serum cholesterol above 350 mg/100 ml and triglycerides (TG) above 3.50 mmol/l at routine determination of serum cholesterol and TG, among about 20 000 subjects at a health control centre. Reference subjects with non-elevated serum lipids were obtained from the same centre. Except for the serum lipids the same criteria for participation were applied to these subjects. Around three months after screening, the hyperlipidaemic and control subjects presented at the Lipid Unit, Department of Internal Medicine, Karolinska Institute, and a second serum lipids analysis together with quantitative estimation of cholesterol and TG concentrations in the three LP fractions: very low (VLDL), low (LDL) and high (HDL) density LP were performed. The sera of all subjects were then typed according to Fredrickson et al. (10) as modified by Alling (11). All determinations were performed at the King Gustaf V Research Institute.

For each sex the individuals with HLP were allocated into 6 different groups according to the

Table 1 Age, body weight, body height, weight/height (W/H) index and body surface area (BSA) in men and women in the different HLP groups and in controls (C) (mean \pm S.E.M. and range)

Group	Age (y)	Body weight (kg)	Body height (cm)	W/H index*	BSA (m ²)
Men					
C	39 48 \pm 1 (28-70)	76 1 \pm 1.4 (50.5-120.5)	178 \pm 1 (16-197)	0.97 \pm 0.02 (0.75-1.44)	1.93 \pm 0.02 (1.56-2.41)
I	35 48 \pm 1 (27-64)	77 1 \pm 1.8 (52.2-111.0)	177 \pm 1 (170-187)	0.99 \pm 0.02 (0.63-1.30)	1.94 \pm 0.02 (1.70-2.09)
II A	31 48 \pm 2 (3-68)	73.5 \pm 1.9 (58.0-97.5)	175 \pm 1 (162-186)	0.98 \pm 0.02 (0.76-1.34)	1.88 \pm 0.02 (1.63-1.99)
II B	14 53 \pm 3 (40-64)	77.3 \pm 1.1 (69.0-84.3)	176 \pm 1 (164-186)	1.02 \pm 0.02 (0.94-1.13)	1.94 \pm 0.02 (1.78-2.08)
III	11 50 \pm 3 (32-67)	76.3 \pm 2.7 (59.3-88.0)	176 \pm 3 (160-188)	1.01 \pm 0.03 (0.81-1.16)	1.92 \pm 0.03 (1.6-2.08)
IV	34 47 \pm 1 (25-70)	82.2 \pm 1.0* (63.0-111.0)	176 \pm 1 (164-193)	1.09 \pm 0.01 (0.83-1.61)	1.98 \pm 0.01 (1.73-2.37)
V	3 42 \pm 6 (36-60)	84.8 \pm 8.0 (74.9-100.5)	180 \pm 1 (178-181)	1.06 \pm 0.09 (0.96-1.44)	2.04 \pm 0.08 (1.97-2.20)
Women					
C	69 5 \pm 1 (24-73)	61.0 \pm 1.0 (46.5-80.0)	165 \pm 1 (15-179)	0.94 \pm 0.01 (0.79-1.33)	1.67 \pm 0.01 (1.47-1.97)
I	31 54 \pm 3 (25-66)	61.9 \pm 1.7 (43.3-89.0)	164 \pm 1 (157-178)	0.99 \pm 0.03 (0.69-1.44)	1.68 \pm 0.02 (1.43-1.94)
II A	46 56 \pm 1 (3-71)	60.5 \pm 1.1 (47.0-82.0)	162 \pm 1 (150-173)	0.97 \pm 0.02 (0.78-1.32)	1.64 \pm 0.03 (1.42-1.95)
II B	12 56 \pm 2 (48-62)	63.3 \pm 0.9 (51.2-89.0)	162 \pm 1 (150-170)	1.02 \pm 0.04 (0.88-1.41)	1.67 \pm 0.04 (1.45-1.94)
III	6 53 \pm 3 (43-64)	72.3 \pm 4.9 (56.0-91.5)	165 \pm 3 (153-177)	1.12 \pm 0.07 (0.90-1.43)	1.79 \pm 0.06 (1.54-1.98)
IV	30 54 \pm 1 (42-63)	68.8 \pm 1.8 (51.5-92.2)	163 \pm 1 (154-175)	1.09 \pm 0.03 (0.70-1.38)	1.74 \pm 0.02 (1.51-2.01)
V	1 41	52.7	163	0.84	1.55

Calculated as $\frac{\text{body weight (kg)}}{\text{body height (cm)}^2 \times 100}$

Calculated as $(\text{body weight (kg)})^{0.725} (\text{body height (cm)})^{0.725} \times 0.007184 (4)$

Normal type at LP determination despite hyperlipidaemia at screening.

*significant difference against control groups at 5 and 0.05 level respectively

ble 1). In spite of the high cut-off points for the initial (screening) serum cholesterol and TG values at the health control centre a number of subjects were classified as having a normal LP pattern based upon the results for serum LP concentrations determined on the sample taken at the Lipid Unit. Further analysis of the data these subjects will be treated as one group of HLP named N.

The interview and examination followed standardized questions re: comprising preceding illness, present cardiovascular symptoms, current drug treatment, family history, smoking and alcohol habits. Persons smoking a pipe or cigars daily at the time of the screening were classified as smokers. Those who had stopped smoking before that occasion but had smoked for at least five years were regarded as ex-smokers. All subjects were asked about their monthly wine consumption of spirit and wine. These figures were converted into grams of alcohol. One bottle (75 cl) of wine and spirit was considered equal to 60 and 250 g absolute alcohol respectively.

Before routine physical examination special attention was directed towards the finding of stigmata of HLP. Arcus corneae was diagnosed as benigne distinct white or grey arc or complete circle could be clearly seen inside the peripheral margin of the cornea. Palpebral xanthelasmata were divided into single and if at least two on eyelids multiple. The presence of tendon xanthomata was assessed by inspection and palpation of typical locations and was divided into solitary and multiple xanthomata.

Body weight was measured without coat and jacket and 1/2 kg was subtracted for the clothes. Body height was determined without shoes. Based on these measurement

weight/height (W/H) index and body surface area (BSA) were calculated.

Except for the ESR determined at the Lipid Unit by the Westergren method in plastic tubes, all non-lipid methods reported here were routine methods of the Department of Clinical Chemistry, Karolinska sjukhuset. Thus no special attention was paid to these determinations, it being part of research project. During the course of the study no major changes of the methods were made and samples from the control subject were interspersed with samples from HLP subjects.

Hb was analysed as cyanmethaemoglobin. S-GOT and S-GPT were determined enzymatically on a reaction rate analyser LKP 8600. Serum uric acid was determined by the uricase method. The glucose tolerance test (IVGTT) was performed in the morning after an overnight fast. The evening before IVGTT the subject were given 50 g glucose by mouth. Twenty-five g glucose was given intravenously. The estimation of the half-life ($T_{1/2}$) of glucose was based on 10 blood glucose determinations of blood samples drawn with 5-min intervals beginning 15 min after the injection. The elimination rate k value was determined according to the equation $k = \frac{0.693}{T_{1/2}}$ (13).

$$k = \frac{0.693}{T_{1/2}} \quad (13)$$

Blood glucose was determined by the glucose oxidase method.

Conventional statistical methods were used for the calculations of the arithmetic mean and S.E.M. For comparison of quantitative data Student's t test (unpaired data) was used. To compare the difference of proportion the Fisher exact probability test (26) was used. To de-

scribe the joint relationship of single variable to one or several other variables regression analysis was applied. In the present paper tests for significant differences between controls and ten HLP groups have been performed for great number of variables. By chance alone 5% of the *p*-values can be expected to be higher than 1.96 or lower than 1.96 even if there were not true differences of any kind between the HLP groups and controls. Statistical significance at the 5% level should therefore be interpreted with caution.

RESULTS

Anthropometric data (Table I)

In men no difference in age was found between the control and HLP groups whereas women with type IIA were older than control women. On an average the females were 3-8 years older than the males. Men and women with type IV HLP were on an average 6 and 8 kg respectively heavier than controls. Mean body height did not differ between these groups and the controls but both men and women with type IIA were shorter than the controls. Mean weight/height index in men with type IIB and in both men and women with type IV HLP was higher than in controls indicating that they were more obese. Mean BSA was larger in men and women with type IV HLP.

Smoking habits (Table II)

Among men there was a higher proportion of smokers in types of HLP characterized by elevated VLDL TG concentrations (types IIB, III, IV and V taken together $p < 0.05$) compared to the control group. The male controls as well as males in all HLP groups smoked about 15 cigarettes daily. The mean number of years smoked and the percentage of inhalers did not differ between controls and HLP

Table II Smoking habits in the different HLP groups and in controls (C)

Group	Smokers (%)	Ex-smokers (%)
<i>Men</i>		
C	39	46
N	33	43
IIA	31	48
IIB	14	86
III	11	64
IV	94	57
V	3	67
<i>Women</i>		
C	69	25
N	31	39
IIA	46	30
IIB	12	33
III	8	17
IV	30	63 **
V	1	100

***=significance at the 5 and 1% level against control group.

N see footnote to Table I

groups. About 10% of the men in each group smoked a pipe. Male exsmokers were most frequently seen in the N group.

Among women with HLP smoking was most frequent in type IV. The mean consumption among the controls and the women in all HLP groups was 18 cigarettes daily.

Arcus corneae

As the occurrence of stigmata of HLP (Table III) did not differ essentially between the sexes the results are given for males and females together divided into different types of HLP and controls. The only HLP stigma found in the control group

Table III Stigmata of HLP (%) in different HLP groups and in controls (C)

Group		<i>Arcus corneae</i>	<i>Xanthelasma</i>		<i>Tendon xanthomata</i>		<i>Palmar xanthomata</i>	<i>Eruptive xanthomata</i>
			All	Multiple	All	Multiple		
C	128	29	0	0	0	0	0	0
N	66	50**	6	0	8	0	0	0
IIA	77	69*	10**	3	43 **	14**	1	0
IIB	26	54	19**	4	3	0	0	0
III	17	29	6	0	0	0	1	0
IV	14	38	2	1	0	0	0	0
V	4	25	0	0	0	0	0	25

***=significant differences against the control group 1:5, 1 and 0.1% level respectively
 ** see footnote to Table I

Table IV Serum lipids, lipoproteins and clinical findings in subjects with xanthomata

Chol = cholesterol (mg/100 ml), TG = triglycerides (mmol/l)

Subj no	Sex	Age (y)	Screening values		Type of HLP	Lipoprotein concentration				LDL		HDL	
			Chol	TG		VLDL		Chol/TG β	Chol	TG	Chol	TG	
						Chol	TG						
Multiple tendon xanthomata													
1	♂	32	453	2.12	II A	15	0.60	.5	—	385	0.80	52	0.37
	♂	34	404	1.64	II A	15	0.45	.33	—	263	0.6	37	0.13
3	♂	35	436	1.4	II A	37	1.14	.3	—	289	0.71	30	0.15
4	♂	35	411	1.44	II A	.3	0.41	.56	—	265	0.74	49	0.21
5	♂	45	501	1.37	II A	18	0.5	.35	—	417	1.13	53	0.23
6	♂	52	356	1.83	II A	11	0.43	.76	—	237	0.41	60	0.20
7	♂	53	416	1.64	II A	22	0.82	.27	—	262	0.73	54	0.24
8	♀	41	468	1.78	II A	23	0.70	.33	—	307	0.71	53	0.16
9	♀	56	394	0.99	II A	5	0.20	.5	—	366	0.44	85	0.18
10	♀	61	502	1.59	II A	77	0.93	.29	—	398	0.70	62	0.44
11	♀	63	458	1.81	II A	25	0.99	.5	—	276	0.60	58	0.28
Palmar xanthomata													
1	♂	56	357	3.31	II A	53	1.18	.45	+	224	0.50	45	0.25
2	♂	57	464	14.10	III	287	10.25	.28	+	99	0.29	38	0.26
3	♂	59	351	5.39	III	119	2.73	.44	+	133	0.62	35	0.22
Erectile xanthomata													
1	♂	36	385	36.6	V	319	64.9	.5	—	49	0.72	2	0.56

Expressed according to the Minnesota code

Ischemia time at digital pulse plethysmography of the lower limbs.

Left bundle branch block.

was arcus corneae present in almost 1/3 of the cases. Subjects with type II A had the highest frequency of arcus followed by the type II B and N groups while it was slightly less common in type IV HLP. A correlation existed between the frequency corneal arcus for each group and its mean LDL cholesterol ($r=0.93$, $p<0.001$). Complete circular was most often seen in types II A (17%) and II B HLP (1%). Xanthelasmata were found in all HLP groups including the N group most frequently in type II B.

Tendon xanthomata

Tendon xanthomata were seen mostly but not exclusively in type II. Three subjects with tendon xanthomata in the N group and in the type IV group had initial serum cholesterol concentrations above 350 mg/100 ml.

Multiple tendon xanthomata (Table IV) were found in 11 subjects exclusively with type II A HLP; they will be called II A X subjects (6). Twenty two per cent of the men and 9% of the women had II A X. Mean age of the 7 II A X men

was 9 years below that of other males with type II A HLP and 4 of them were below 36 years of age. The mean age of II A X women was similar to that of the other type II A women. Initial serum cholesterol concentrations were above 400 mg/100 ml in all but two of the II A X subjects. In nine II A X subjects initial serum TG was below 2.00 mmol/l. In type II A X men mean initial serum cholesterol was around 50 mg/100 ml higher than that of the other subjects with type II A. The corresponding figure for women was around 100 mg/100 ml.

For both male and female II A X groups the mean total serum cholesterol concentrations decreased from the first (screening) to the second (LP analysis) determination to the same degree as for the whole II A groups, i.e. around 10% for the males and 6% for the females. The higher total cholesterol concentrations in II A X subjects were attributable to higher LDL cholesterol concentrations in this condition as neither VLDL cholesterol nor HDL cholesterol differed greatly between II A X and the other type II A subjects. In both male and female II A X groups initial serum TG was lower than in the remaining type II A population.

Intermittent claudication among relatives of II A X individuals was known in two cases above the age of 60

Exercise ECG ST ^a depression	TT ^a (caec)	ESR (mm/h)
0	9.5	11
4.1	11.5	9
0	10.0	34
0	10.0	10
0	11.0	1
4.3	10.8	8
4.7	11.0	23
4.2	10.5	15
4.1	10.0	18
4.7	12.5	9
✓	12.0	8
8	10.5	18
4.1	11.8	10
4.4	17.8	48
4.3	9.5	21

This corresponded to a lower VLDL TG concentration in II A X. The cholesterol/TG ratio in VLDL was often high in these subjects (19)

The prevalence of ST segment depressions during exercise ECG (20) and signs of peripheral vascular disease at digital pulse plethysmography (21) were assessed in all HLP subjects. Two of the seven men and two of the four women with II A X had "ischaemic" ST segment depressions during exercise (4 1-4 4 according to the Minnesota code). The oldest type II A-X woman had a left bundle branch block.

None of the II A X individuals had pathological digital pulse plethysmogram in the study of the peripheral circulation indicating absence of signs of peripheral vascular disease

The cause of death of the 22 parents of II A X subjects was known in 12 cases. Six of them had died from myocardial infarction, the fathers ($n=5$) at a mean age of 70 and the only mother at an age of 83. Seven cases of myocardial infarction were known among other relatives: three of them before the age of 60. Angina pectoris was also known in seven cases: in five of them before the age of 60.

Palmar and eruptive xanthomata

Two type III males and one male with serum typed as II A HLP had palmar xanthomata (Table IV). Both the serum cholesterol and TG of the latter subject were elevated at screening. By the time of the LP determination his total TG had however decreased to 28 mmol/l and the VLDL TG concentration was below our arbitrary upper normal limit (1.25 mmol/l). LDL cholesterol was above the upper normal limit (220 mg/100 ml). VLDL cholesterol was 53 mg/100 ml giving a high VLDL cholesterol/TG ratio. Electrophoresis showed a "floating β -LP". This subject thus had a clinical picture of type III HLP but his serum was typed as II A in spite of the presence of a "floating β -LP". This was a consequence of the current criteria for classification of HLP (1) as his VLDL TG were not elevated while LDL cholesterol was. Two of the subjects with palmar xanthomata had ST segment depressions during exercise and one had a pathological pulse plethysmogram indicating subclinical peripheral vascular disease.

One of the type V subjects had eruptive xanthomata of elbows and buttocks. His exercise ECG showed ST segment depressions.

Laboratory findings (Table V)

Men with type IV and women with type IIB HLP had higher Hb concentrations than controls. Thus increased Hb concentrations were seen in HLP types characterized by elevated VLDL TG concentrations. No correlation was found between VLDL TG and Hb concentration.

Mean ESR was higher in males with types II A, IIB and IV HLP and in females with types N, II A, IIB, III and IV HLP.

S-GOT was in general not elevated in HLP. Type IV HLP S-GOT was elevated compared with the controls in both men and women (Fig. 1). Because of the skewed distribution of S-GOT a significance test was also performed on logarithms of S-GOT. The results were equal. This increase was due partly to a shift of the whole distribution of type IV S-GOT towards higher levels and partly to a great number of markedly elevated S-GOTs in type IV men and women. The decrease of S-GOT on age, body weight, alcohol

Table V Laboratory findings in subjects with different types of primary HLP and in controls (C) (means \pm S.E.M.)

Group	n	Hb (g/100 ml)	ESR (mm/h)	S-GOT (U/l)	S-GPT (U/l)	Uric acid (mg/100 ml)	IVGTT	
							Δ %/h	% < 1.10*
Men								
C	59	14.8 \pm 0.1	9.7 \pm 1.0	11.5 \pm 1.0	9.8 \pm 0.9	5.1 \pm 0.1	—	—
N	35	15.1 \pm 0.2	12.1 \pm 1.5	12.7 \pm 1.8	10.3 \pm 0.8	6.0 \pm 0.1**	1.33 \pm 0.09	31
II A	31	14.5 \pm 0.1	17.9 \pm 2.6	10.2 \pm 1.0	8.9 \pm 0.6	5.6 \pm 0.3	1.39 \pm 0.10	32
II B	14	14.8 \pm 0.3	31.3 \pm 6.6	10.3 \pm 0.9	10.6 \pm 1.3	5.2 \pm 0.3	1.45 \pm 0.19	36
III	11	14.6 \pm 0.4	17.4 \pm 4.6	9.7 \pm 0.4	10.5 \pm 1.5	5.0 \pm 0.2	1.34 \pm 0.13	45
IV	94	15.1 \pm 0.1	13.3 \pm 1.3	11.6 \pm 0.5	10.4 \pm 0.8	6.0 \pm 0.1**	1.24 \pm 0.05	43
V	3	15.7 \pm 0.2	33.7 \pm 9.1	12.0 \pm 1.2	11.3 \pm 1.2	5.9 \pm 0.4	1.02 \pm 0.12	87
Women								
C	69	13.5 \pm 0.1	12.0 \pm 1.1	8.5 \pm 0.3	7.2 \pm 0.3	3.7 \pm 0.1	—	—
N	31	13.8 \pm 0.2	19.3 \pm 3.5	9.8 \pm 0.7	8.6 \pm 1.1	4.1 \pm 0.2	1.73 \pm 0.08	3
II A	46	13.6 \pm 0.1	22.7 \pm 2.1	9.5 \pm 0.4	7.3 \pm 0.4	3.9 \pm 0.1	1.51 \pm 0.09	20
II B	1	14.1 \pm 0.2	28.0 \pm 4.7*	8.9 \pm 0.7	10.1 \pm 1.9	4.3 \pm 0.4	1.33 \pm 0.16	30
III	6	13.6 \pm 0.5	30.0 \pm 4.4	11.2 \pm 1.7	10.3 \pm 1.9	5.3 \pm 0.4	1.49 \pm 0.19	17
IV	30	13.9 \pm 0.1	4.9 \pm 2.6	11.3 \pm 0.8*	11.1 \pm 1.0*	4.9 \pm 0.2	1.47 \pm 0.11	30
V	1	15.0	11	7.6	18	4.8	2.46	0

* = significant differences against C group at 5% and 0.1% levels respectively

Percentage of subjects with diabetic or borderline glucose tolerance.

N = see footnote to Table I.

TG and serum uric acid concentration was studied by multiple regression analysis. In type IV males S-GPT was significantly negatively correlated to age and positively to VLDL TG. In females with type IV and male and female control groups no correlations were found.

Uric acid was increased in male N group and in the male and female groups with type IV. This was further studied by regression analysis using age, body weight, VLDL TG and S-GPT as independent variables. In both men and women (controls + all HLP) the serum uric acid concentration was dependent on body weight ($p < 0.01$) and $p < 0.005$ respectively). In addition in females II was also dependent on VLDL TG ($p < 0.005$) and alcohol intake ($p < 0.01$). If the same calculation was applied to type IV males only, VLDL TG was significantly correlated with serum uric acid ($p < 0.02$).

IVGTT

For practical reasons IVGTT could be performed only in subjects with HLP. No significant differences of mean Δ values were found between different HLP groups. The proportion of pathologic and borderline Δ values tended to be higher in groups with VLDL TG elevation.

DISCUSSION

In the different HLP groups men were generally 3–8 years younger than women. This was probably in part due to the higher age of the women attending the health control centre. The mean age of women was approximately two years above that of men in the basal health control population. In addition selective mechanisms could partly explain these age differences within the HLP sample as it is well known that in general ischaemic heart disease in men precedes that of women by several years (15). All subjects with overt cardiovascular disease were excluded from this study.

HLP subjects with VLDL TG elevation were more obese than controls. This is in agreement with the clinical fact that subjects with hypertriglyceridaemia are often moderately overweight. In this study both male and female type II A subjects weighed less than controls. This corresponded to a shorter mean body height in this type of HLP.

The percentage of smokers in male and female control groups is in agreement with investigations on smoking habits of the populations of corresponding age groups in large Swedish cities (28). The proportion of male smokers in the groups with elevation of VLDL TG (type II B, III, IV and V) was

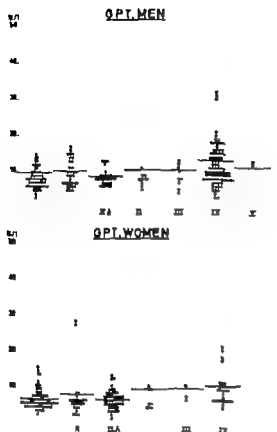


Fig. 1 OPT: men and women with different types of hyperlipoproteinaemia and in controls (C) with non-elevated serum lipids. N = subjects with normal lipoprotein pattern in spite of hyperlipidaemia at screening. The horizontal lines indicate mean values.

higher. In this city the serum TG levels for male and female smokers have been shown to be higher than for non-smokers (7). Similarly Gofman *et al.* (11) reported an elevation of VLDL in young men smoking more than 20 cigarettes a day. One possible mechanism behind this finding is that smoking increases the mobilization of free fatty acids (FFA) from adipose tissue (14). FFA could in turn be extracted by the liver (3), incorporated into liver TG and secreted as VLDL TG (14). Another possibility is that smokers and non-smokers have constitutional differences which also influence the serum TG concentrations.

The occurrence of stigmata of HLP in different types has been extensively reviewed before (27). The most prevalent HLP stigma seen in this study was arcus corneae. It was also seen to a great extent in subjects with non-elevated serum lipids. It could

therefore be questioned whether arcus corneae really should be called a HLP stigma. Its association with serum lipids has also been questioned (9, 16, 23). However, as there was a highly significant correlation between mean LDL cholesterol for each HLP group and for the controls and the frequency of corneal arcus, it is conceivable that the concentration of LDL cholesterol has a direct influence on the deposits in the cornea. It seems therefore justifiable to call arcus corneae a HLP stigma, but the 'threshold LDL cholesterol concentration—if any—for arcus formation must be much lower than the arbitrarily chosen cut-off point for type II HLP. The clinical importance of arcus has furthermore been emphasized recently (25) by the finding that it is a risk factor for coronary heart disease independent of both serum cholesterol and age.

Also palpebral xanthelasmata were found in moderate LP elevation of different types but most often in types II A and II B.

In contrast to arcus and xanthelasmata, HLP stigmata like tendon xanthomata, palmar xanthomata and eruptive xanthomata were relatively rare and were accompanied by high LP concentrations. Multiple tendon xanthomata were only seen in type II A HLP.

Eleven subjects with type II A HLP had multiple tendon xanthomata (II A X). Studies from Scandinavia have shown that tendinous xanthomata are associated with hypercholesterolaemia and premature ischaemic heart disease (12, 17). The males with II A X in the present study were younger than other type II A males. The reason for this was probably that older II A X men already had developed symptoms of cardiovascular disease and thus had been excluded from this asymptomatic sample of HLP subjects. However, the oldest II A X man was 55 and the oldest woman 63 years of age, indicating that marked hypercholesterolaemia could be asymptomatic up to a relatively old age. Signs of coronary artery disease were frequent in this group (20), while signs of peripheral vascular disease were absent (21). Half of the deceased parents of II A X subjects had died from myocardial infarction. These findings are probably consistent with a genetic transmission of type II A X.

One case with eruptive xanthomata was seen in conjunction with extreme VLDL TG elevation. Palmar xanthomata (3 cases) were only seen in presence of 'floating β -LP' though one as II A HLP. Based on this material and

ence from other studies (10-12) HLP stigmata found at a health control might therefore be divided into *general* (arcus corneae xanthelasmata) and *diagnostic* (multiple tendon xanthomata=type IIA palmar xanthomata=type III HLP and eruptive xanthomata=type V or severe type IV HLP).

Men with type IV and women with type IIB HLP had higher Hb concentrations than controls. The differences observed in this study between HLP and control groups are small and inconclusive. Increased Hb concentrations were only seen in groups with increased VLDL TG concentrations. As Hb is determined spectrophotometrically and VLDL absorbs light, high VLDL concentrations might give rise to falsely high readings. This might at least in part explain the higher Hb values seen in HLP.

The high ESR found in HLP is discussed elsewhere (5) and is not believed to be caused by the LP interfering with ESR but to reflect underlying vascular disease.

Both men and women with type IV HLP had higher mean S-GPT values than controls. This might reflect a higher extent of damage to the liver cells in type IV HLP. Such damage might reflect a greater exposure to agent affecting the liver on the one hand and on the other a greater susceptibility of the liver cells to those agents in type IV HLP. One possible mechanism for the first alternative is an increased alcohol consumption among type IV subjects.

Among men with type IV HLP there was a higher proportion of subjects (51%) consuming 240 g alcohol or more monthly than among controls (37%) ($p < 0.01$). Among women with type IV HLP there was an insignificantly higher proportion of subjects stating a consumption of 1-3 g alcohol or more monthly than among control. However no correlation existed either between alcohol intake and VLDL TG or alcohol intake and S-GPT. Therefore no cause-effect relationship could be established between alcohol consumption and elevated VLDL TG concentrations or liver damage in the present study.

Serum TG levels are reported to be elevated in gout (8). In subjects selected on account of this diagnosis no correlation existed between serum TG and serum uric acid levels. Berkowitz (1) reported higher uric acid levels in patients with hypertriglyceridaemia and a significant correlation between serum TG and uric acid levels in these subjects. Furthermore a positive association between

alcohol consumption and elevated serum uric acid level is well known (18).

Although all subjects with gout were excluded in the present study (19) both male and female type IV groups had increased serum uric acid concentrations when compared to the control groups. This was further analysed by multiple regression. The uric acid level was significantly related to body weight and VLDL TG concentrations, but not to age. In females it was also correlated to the stated alcohol intake. This suggests that the uric acid concentration independently is determined by several mechanisms including body weight, alcohol consumption and VLDL TG in subjects with HLP.

ACKNOWLEDGEMENT

Supported by grants from the Swedish National Association against Heart and Chest Diseases.

REFERENCES

- 1 Beaumont, J. L., Carlsson, L. A., Cooper, G. M., Fajfar, Z., Fredrickson, D. S. & Strasser, T. Classification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. World Health Org.* 43: 891, 1970.
- 2 Berkowitz, D. Blood lipid and uric acid interrelationships. *J. A. M. A.* 190: 856, 1964.
- 3 Boberg, J., Carlsson, L. A. & Freylich, U. Determination of splanchnic secretion rate of plasma triglycerides and of plasma free fatty acid total and splanchnic turnover. *Europ. J. Clin. Invest.* 2: 123, 1972.
- 4 Du Bois, D. & de Bois, E. F. A formula to estimate the approximate surface area if height and weight be known. *Arch. Intern. Med.* 17: 863, 1916.
- 5 Böttiger, L. E., Carlsson, L. A., Ekelund, L. G. & Olsson, A. G. Raised erythrocyte sedimentation rate in asymptomatic hyperlipidaemia. *Brit. med. J.* 68: 1, 1973.
- 6 Carlsson, L. A. Serum lipoprotein composition in different types of hyperlipoproteinaemia. In: Proceedings of the V International Symposium on Drugs Affecting Lipid Metabolism, 1974 (ed. W. L. Holmes, D. Kritschovsky & R. Paoletti). In press, 1975.
- 7 Carlsson, L. A. & Lindstedt, S. The Stockholm Prospective Study I. The initial values for plasma lipid. *Acta med. scand. Suppl.* 493: 1969.
- 8 Feldman, E. H. & Wallace, S. L. Hypertriglyceridaemia in gout. *Circulation* 29: 508, 1964.
- 9 Forss, H. Arcus senilis corneae: its clinical development and relationship to serum lipids, proteins and lipoproteins. *Acta ophthalm. Suppl.* 42: 1, 1954.
- 10 Fredrickson, D. S., Levy, R. I. & Lees, R. S. Fat transport in lipoproteins, an integrated approach to mechanism and disorders. *New Engl. J. Med.* 276: 34-44, 1967.

11. Gofason, J. W., Lindgren, F. T., Strisower, B. de Lalla, O., Glazier, F. & Tamplin, A. Cigarette smoking, serum lipoproteins and coronary heart disease. *Geriatrics* 10: 349, 1955.
12. Harbitz, F. Über plötzlichen Tod mit natürlichen (d. h. nicht gewaltsamen) Todesursachen insbesondere bei jungen Leuten. Monograph 5 Norsk videnskabsakademi (Oslo 1938).
13. Ilkos, D. & Luft, R., On the intravenous glucose tolerance test. *Acta endocr. (kbb.)* 25: 31., 1957.
14. Kershbaum, A., Bellet, S., Dickstein, E. R. & Feinberg, L. J. Effect of cigarette smoking and nicotine on serum free fatty acids based on study of the human subject and the experimental animal. *Circulat. Res.* 9: 631, 1961.
15. Kannel, W. B., Kagan, A., Dawber, T. R. & Revotzky, N. Epidemiology of coronary heart disease. *Geriatrics* 17: 675, 1962.
16. McAndrew, G. M. & Ogston, D. Arcus senilis in middle-aged men. *Brit. med. J.* 1: 425, 1965.
17. Miller, C. Xanthomata, hypercholesterolaemia angina pectoris. *Acta med. scand., Suppl.* 89: 75, 1938.
18. Myrnes, M., Alcohol consumption in relation to factors associated with ischaemic heart disease. *Acta med. scand., Suppl.* 367, 1974.
19. Olsson, A. G. & Carlsson, L. A. Studies in asymptomatic primary hyperlipidaemia. I. Types of hyperlipoproteinaemias and serum lipoprotein concentrations, compositions and interrelations. *Acta med. scand. Suppl.* 580, 1, press 1975.
20. Olsson, A. G., Eklund, L. B. & Carlsson, L. A. Studies in asymptomatic primary hyperlipidaemia. IV. ECG at rest and during exercise and its relation to various lipoprotein classes. *Acta med. scand.* 1, press 1975.
21. Olsson, A. G. & Eklund, B. Studies in asymptomatic primary hyperlipidaemia. V. Peripheral circulation. *Acta med. scand.* In press 1975.
22. Polano, M. K., Xanthomatosis and hyperlipoproteinaemia. *Dermatologia* 149: 1, 1974.
23. Rallid, B. M. The incidence of arcus senilis in ischaemic heart disease: its relation to serum lipid levels. *Lancet* 1: 312, 1965.
24. Robinson, D. S. The function of the plasma triglycerides in fatty acid transport. *Comprehensive biochemistry. Lipid metabolism* (ed. M. Florin and E. H. Stoltz) chapter 1, p. 51. Elsevier Amsterdam 1970.
25. Roseman, R. H., Brand, R. J., Sholtz, R. I. & Jenkins, C. D. Corneal arcus, cardiovascular risk factors and coronary disease. *New Engl. J. Med.* 291: 1322, 1974.
26. Siegel, S. Nonparametric statistics. International student edition. McGraw-Hill New York, Toronto and London.
27. Stambury, J. B., Wyngaarden, J. & Fredrickson, J. S. The metabolic basis of inherited disease 3rd ed. McGraw-Hill New York, Toronto and London 1972.
28. Tobaksrökning. En rapport till socialstyrelsen tobaksreduktion. Allmänna Förlaget, Stockholm 1973.

STUDIES IN ASYMPTOMATIC PRIMARY HYPERLIPIDAEMIA

III Physical Working Capacity

Lars-Göran Ekelund and Anders G. Olsson

*From the Department of Clinical Physiology and Internal Medicine (Lipid Unit)
Karolinska sjukhuset and King Gustaf V Research Institute Stockholm, Sweden*

ABSTRACT One-hundred-and-sixty male and 123 female subjects with asymptomatic primary hyperlipidaemia (HLP) selected from a health control centre have been studied with a heart rate (HR) controlled exercise test. The serum cholesterol and/or TG at screening were above 350 mg/100 ml and 3.5 mmol/l, respectively. As a reference group 49 male and 60 female age-matched subjects from the same centre with serum cholesterol below 300 mg/100 ml and TG below 2.00 mmol/l were investigated using the same technique. Quantitative lipoprotein (LP) analyses and typing were performed on all HLP and control subjects. The subjects were divided into two age groups: 35-50 and above 50 years of age. Men with all types of HLP had lower working capacity expressed in W_{170} or W_{180} than controls, most pronounced in the younger age group. Younger women with type II A had lower working capacity than their controls. After correction for variation in body weight and age there remained a significantly lower W_{180} in male types II A (11%) and IV (21%) and female type II A (11%). There was no difference in systolic BP during exercise between controls and HLP. The total exercise time and final HR did not differ in control and HLP subjects. Dynamic spirometry was performed in 103 of the male subjects and a significantly lower vital capacity was found in HLP subjects compared with controls after correction for variation in age, weight and height. No differences were found in the maximal flow values. The observed differences in working capacity between controls and HLP subjects are explained by a difference in stroke volume. The lower stroke volumes in male types II A and IV and female type II A could be explained by a lower degree of physical fitness, by a common genetic factor resulting in HLP and decreased stroke volume or by a less effective myocardial function in HLP subjects.

Hyperlipoproteinaemia (HLP) has been shown to be of importance for the development of ischaemic

heart disease (IHD) in many clinical and epidemiological studies. The mechanism behind this association is most probably through an increase in coronary atherosclerosis promoted by HLP.

There is an association between serum lipids and physical activity. Physically active subjects have lower cholesterol and triglyceride (TG) levels than others (6-8, 11). Middle-aged runners are reported to have lower serum TG and low density lipoprotein (LDL) cholesterol concentrations but higher high density lipoprotein (HDL) cholesterol concentrations than others (28). Very little is however known about the physical working capacity in subjects with hyperlipidaemia.

In a study of asymptomatic subjects with primary HLP (7) an exercise ECG was performed to determine the frequency of ST segment depressions during exercise. In the present study the physical working capacity was evaluated in these subjects as the design of the exercise test also permitted a good estimation of the functional capacity. The results were compared with a reference group with non-elevated serum lipids. Quantitative lipoprotein (LP) analysis was performed on all HLP and control subjects in order to relate differences in working capacity to LP concentrations.

SUBJECTS AND CHEMICAL METHODS

The sampling procedure and the method for serum lipid and LP determinations have been reported elsewhere (21). In essence serum lipids were analyzed on about 7000 subjects attending health control centre. Two-hundred and eighty-three subjects above 35 years with serum cholesterol levels above 350 mg/100 ml and/or serum TG above 3.5 mmol/l but without diagnosis or symptoms of secondary HLP cardiovascular disease, diabetes or

Table 1 Anthropometric data in subjects with different types of HLP and in controls (C)

Men				Women		
Group	n	Body weight (kg)	Body height (cm)		Body weight (kg)	Body height (cm)
C	49	74.8±1.4	178±1	60	61.0±0.9	165±1
N	30	76.2±1.7	177±1	30	61.9±1.6	163±1
II A	76	72.9±1.1	175±1	45	59.8±1.1	161±1
II B	14	76.0±1.3	176±1	12	62.5±2.9	166±2
III	9	75.3±3.3	175±3	6	71.7±4.3	165±3
IV	11	79.8±1.0*	175±1	30	68.0±1.8	163±1

* = significant differences against the control groups at the 5% and 0.1% levels, respectively.

N indicates subject with normal LP type despite hyperlipidaemia at screening.

hypertension were investigated none was on chronic drug treatment. One-hundred-and-nine age-matched male and female control subjects from the same health control centre with serum cholesterol below 300 mg/100 ml and TG below 0.0 mmol/L were offered the same investigations. Except for the serum lipids the same criteria for participation were applied to them. The number of and reasons for exclusions of HLP and control subjects are given elsewhere (21).

About three months after the hyperlipidaemia had been diagnosed in a subject the fasting concentrations of cholesterol and TG were determined in each of the very low (VLDL) low (LDL) and high (HDL) density LP class after separation by preparative ultracentrifugation (7). LP paper electrophoresis was run on whole serum and on top and bottom fractions after separation ($d=1.006$). Typing of HLP was performed according to Fredrickson et al (1) as modified by Beaumont et al (8).

All HLP and control groups were divided into two age groups: 36–50 (younger group) and above 50 years (older). In none of the HLP groups did mean age differ significantly from that in the control group. In spite of the cut-off point for the initial (screening) serum cholesterol and TG values at the health control centre, number of subjects were classified as having normal LP pattern based upon the results for serum LP concentrations determined on the sample taken at the Lipid Unit. The possible reasons for this have been discussed (21). In the further analysis of data these subjects will be treated as a separate HLP group named N.

All subjects were interviewed according to a standardized questionnaire including subjects' estimate of physical activity at work. If they stated that they had had regular physical training sufficient to cause breathlessness at least once a week for the last year they were regarded as physically active subjects.

PHYSIOLOGICAL METHODS

Exercise tests were performed in the sitting position on an electrically braked bicycle ergometer (EM 370 Siemens-Elmab, Sweden) controlled by a heart rate (HR) controller unit (EMT 572, Siemens-Elmab) which automatically adjusted the work load of the bicycle so that the pre-

selected HR during work could be obtained (9–10). By this method direct measures instead of an intra- or extrapolated was achieved for the work performed at a HR of 130 (W_{130}), 150 (W_{150}) and 170 (W_{170}) beats/min. W_{150} was used as an expression for working capacity. The subjects exercised for 6 min on consecutive loads beginning with a HR of 90 beats/min in individuals above 56 years of age and with 110 beats/min in younger subjects, increasing every 6 min by 20 beats/min. The exercise was continued until fatigue or in a few cases earlier if marked ECG changes were observed. The work load and HR were continuously recorded on an ink-pen recorder (Servogor) and the total work performed was calculated by electrical integration. The ECG recordings comprising 6 limb leads and 6 chest leads (CR) were taken before and after exercise. During exercise 6 chest-lead leads (CH) were recorded continuously. The ECG was recorded on an ink-jet recorder (Mingograf 61) with a time constant of 3 sec and a frequency response up to 700 Hz. The same ergometer was used during the whole study and the calibration of the ergometer was regularly checked. The performance of the ECG recorder was also checked several times with respect to frequency response and accuracy of paper speed. The diagnostic ECG was recorded at a paper speed of 50 mm/sec. The ECG at rest and during exercise was interpreted by one of us (L.-G.E.) without knowledge of whether the ECG was recorded from a control or hyperlipidaemic subject. A magnifier (5 times) was used for the visual interpretation of the ECG recorded at paper speed of 50 mm/sec and the ECG changes were coded according to the Minnesota criteria adopted for CR leads (23). BP was measured in the left arm with the cuff method using calibrated manometers.

Before exercise resting ECG, HR and the systolic and diastolic BPs were noted with the patient in the supine position after 10 min rest. During exercise systolic BPs were measured during the 5 min at each HR level. Breathing frequency was counted during the 3 min between resting ECG and exercise. Standardized orthostatic test was performed with 8 min standing and recording of HR, ECG and BPs after 8 min.

Ventilatory tests were performed in 77 male HLP and 30 male control subjects with spirometer as described by Bernstein et al (4) modified to give an electrical signal output. This signal was analyzed by a computer program.

on an IBM 1800 and values for static vital capacity (VC) including forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV₁) and maximal voluntary ventilation are obtained together with corresponding normal values derived from age, sex, height and weight (3-14).

A chest X-ray with determination of relative heart volume (in standing position) was performed within the clinical routine of the hospital.

Statistical methods according to Snedecor and Cochran (27), Siegel (24) and Hald (15) were used and the data were processed on a computer model Hewlett-Packard 2000E. In the present paper tests for significant differences between controls and several HLP groups divided into three age categories have been performed for a number of variables. By chance alone 5% of the *t* values can be expected to be higher than 1.96 or lower than -1.96, even if there are no true differences of any kind between groups of HLP and controls. Statistical significance at the 5% level should therefore be interpreted with caution.

RESULTS

Anthropometric data The relevant anthropometric data are given in Table I. The results are discussed elsewhere (20).

Physical activity (Table II) No significant difference in stated regular physical activity during time off work was noted in males. Control women stated regular physical activity significantly more often than any HLP group except type III.

Circulatory data

At rest Mean resting HR and systolic and diastolic BP in the different groups are given in Table III. Men with type IV HLP in the younger group had higher resting HR than controls. In this group of HLP there was an inverse correlation between resting HR and working capacity (W_{100}) and the higher resting HR in type IV men might be fully related to their lower working capacity. The same group of HLP subjects also had a higher systolic BP than controls. No correlation between resting HR and systolic BP within this particular HLP group was found. However, a correlation was found between mean resting HR and mean resting systolic BP in the different HLP groups of 36-50-year-old men ($r=0.71$) (Fig. 1) indicating that the increase in BP was parallel to the increase in HR. Women with type IIB and aged above 50 years had higher systolic and diastolic BPs than the controls, which was independent of HR.

Orthostatic test The mean standing HR and systolic BP and the difference in HR and systolic BP

Table II Percentage of subjects stating regular physical activity

Group	Men	Women
C	41	32
N	50	10*
II A	31	13
II B	14	0*
III	22	0
IV	30	6

Symbols as in Table I

between standing and supine positions are given in Table IV. Men with type IV HLP had a higher standing HR than the controls, thus being related to the higher HR at rest. The orthostatic reaction evaluated by the change in HR and systolic BP from supine to standing did not differ between the different HLP groups compared with the control groups.

The prevalence of ST-T changes during the orthostatic test (22) was higher among males with type IV HLP than among controls. This could mean mainly the smallest changes in T (code 1-4) which was consistent with the higher HR in this group (25).

Exercise test The work loads at different HR in controls and HLP subjects are given in Table V. Men with all types of HLP had lower mean working capacity than controls. This difference in working capacity was most pronounced in the younger age groups. Women with type IIA had lower working capacity than the controls.

Total exercise time, final load (W_{end}) and final HR (HR_{end}) are given in Table VI. The total exercise time did not differ significantly between controls and the different HLP groups. W_{end} was significantly lower than in the controls in all men with HLP except type III and in women with types N, II A and IIB HLP. HR_{end} did not differ essentially between the control and HLP groups of either men or women.

As W_{100} , W_{120} and W_{140} were very closely related to each other and therefore only W_{100} will be commented on below. The values for W_{100} were directly obtained in 96% of all subjects. In the remaining 4% extrapolated values were used.

Using multiple stepwise regression and partial correlation there was a significant correlation between W_{100} and body weight together with age both in men and women. No significant correlation was seen between W_{100} and heart volume. If corrections

Table III. Resting HR (beats/min) and systolic and diastolic BP (mmHg) in men and women divided into age groups and types of HLP and in controls (C)

Group	Age (y)	Men			Systolic BP			Diastolic BP		
		Resting HR								
		All	36-50	>50	All	36-50	>50	All	36-50	>50
C		68±1	67±2	68±2	129±	125±2	134±4	82±1	82±2	82±2
n		49	76	23						
N		70±2	71±3	70±3	133±3	128±4	138±3	80±3	80±3	80±
n		30	14	16						
II A		71±	70±3	71±3	179±3	127±4	130±5	82±2	80±3	83±
n		76	11	15						
II B		73±3	78±7	68±3	135±4	128±4	141±5	81±	78±3	83±3
n		14	6	8						
III		70±3	69±6	71±4	133±	127±3	136±	86±2	83±4	88±3
n		9	3	6						
IV		76±	77±	74±3	136±	135±	137±3	85±1	86±1	87±2
n		81	47	34						

Symbols as in Table I

were made for the correlation of \dot{V}_{O_2} to body weight and age eliminating the influence of those factors there was a significantly lower working capacity in males of types II A and IV 109 and 713 kpm/min (11%) (17%) respectively than in controls. Also males of type II B and III had numerically lower \dot{V}_{O_2} 131 and 131 kpm/min. Among the females only type II A had significantly lower \dot{V}_{O_2} 66 kpm/min (11%) after the same corrections for body weight and age.

The systolic BP during exercise did not differ significantly between male or female controls and type II subject although type III had numerically lower values during exercise (Fig. 1).

The respiratory rate at HR of 130 beats/min was significantly higher in the older male group with type II B (18 breaths/min) compared to controls (14 breaths/min) in spite of 17% lower work load.

Ventilatory data (male). Vital capacity (VC) was 89.5% of the expected value for all HLP subjects compared to 97.8% for the control subjects, a significant difference ($p < 0.01$). The difference was significant for types II A, II B and IV and was most pronounced in type II B VC 85.1% of expected value ($p < 0.01$). A significant difference was found in VC between smokers and non-smokers in type IV ($p < 0.01$) but not in the other types or the control group. Forced expiratory volume in one sec (FEV₁) or FEV₁% did not differ between any of the HLP groups and the controls.

DISCUSSION

In our study we have regularly calibrated the ergometer used and standardized with regard to the relative work intensity and duration of exercise using the HR-controlled equipment. As HR is an expression of the relative work intensity provided that the maximal HR is the same (1) we thus have provided approximately the same relative intensity for all age groups as the older subjects worked at a lower absolute HR.

The oxygen transport capacity or physical working capacity is determined by three hemodynamic factors: stroke volume, arteriovenous oxygen dif-

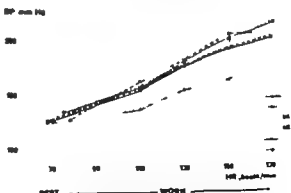


Fig. 1. Mean systolic BP in relation to HR at rest and during exercise in 36-50-year-old men with different types of HLP and in controls.

	mg HR		Systolic BP			Diastolic BP		
	36-50	>50	All	36-50	>50	All	36-50	>50
1	73±2 1	73±2 39	127±2	120±4	130±3	78±1	74±2	80±1
2	73±5 11	75± 19	131±3	128±6	133±4	81±1	83±3	79±2
3	81±4 8	74± 37	133±3	126±7	135±4	80±1	83±4	80±1
4	78±4 4	77±5 8	143±7	126±15	152±6**	84±2*	78±3	87±2*
5	65±8 3	76±8 3	128±11	110±6	147±16	78±4	72±4	83±6
6	73±4 9	77±2 21	128±3	123±4	130±3	79±	75±5	81±2

ference and HR (1.5-17.26). In the present study we have determined working capacity as the work load which gives a HR of 150 beats/min W_{150} . As HR is kept constant, the variation in W_{150} is thus induced by a variation in either stroke volume or arteriovenous oxygen difference or in both. The size of the stroke volume is mainly dependent on the body dimensions (body weight, heart volume, blood volume) and the degree of physical fitness provided that the myocardial function is normal and

not depressed by any disease (5, 6). Arteriovenous oxygen difference at a certain HR is rather constant for different individuals but lower than normal values are found in persons with high activity of the sympathetic nervous system (16) or to a lesser degree in untrained individuals. W_{150} has been found to be a good indirect measure of stroke volume with a reservation for a change in arteriovenous oxygen difference and provided that the exercise test is carefully standardized (17).

Table IV HR (beats/min) and systolic BP (mmHg) in standing position and the difference between standing and supine positions (Δ HR and Δ syst.BP)

Group	Age (y)	HR standing			Δ HR standing-supine			Syst. BP standing			Δ syst. BP standing-supine		
		All	36-50	>50	All	36-50	>50	All	36-50	>50	All	36-50	>50
Men													
C		81±2	81±2	81±2	14±1	14±	13±2	131±3	128±4	134±4	2±	3±4	1±2
N		84±2	84±3	83±3	13±2	13±3	13±2	130±3	125±4	134±4	-2±2	0±	-3±3
II A		83±3	82±3	85±4	13±1	12±2	15±2	131±4	133±4	127±6	2±2	8±4	-3±
II B		87±4	92±7	84±4	15±1	13±1	16±2	133±4	125±5	139±6	-±2	3±4	-1±4
III		90±3	89±6	90±4	20±2	20±1	19±3	131±4	127±6	133±4	-±4	0±3	-3±6
IV		88±2**	90±2*	86±3	12±1	12±1	12±1	135±2	131±	140±3	-1±1	-4±1	3±2
Women													
C		89±2	90±3	89±2	15±1	15±2	16±1	177±3	116±3	133±3	0±2	-4±4	2±2
N		87±3	87±5	87±3	13±	14±	13±3	129±2	130±5	129±3	-2±2	2±3	-4±2
II A		87±	88±4	87±2	11±1	7±3	12±2	131±3	128±6	132±4	-±2	2±3	-3±2
II B		90±4	92±5	89±5	13±2	14±3	12±3	138±5	125±12	144±4	-6±3	-1±6	-8±4
III		88±7	86±12	89±10	16±	20±	13±	128±10	118±13	135±14	-6±5	3±8	-1±4
IV		90±2	88±4	90±3	14±1	15±3	13±1	126±4	119±5	129±4	-±2	-3±2	-2±3

Symbols as in Table I

Table V Physical working capacity expressed as work load (kpm/min) at a HR of 130 beats/min (W_{130}), 150 beats/min (W_{150}) and 170 beats/min (W_{170})

Group	Age (y)	Men								
		All	36-40	>40	All	36-50	>50	All	36-50	>50
C		815±35 49	879±53 26	766±43	1034±44 48	1079±66 26	981±56 22	1263±60 40	1321±85 24	1176±76 16
N		706±30 29	704±40 14	708±45 15	918±35 29	893±49 14	941±51 15	1174±43 25	1099±59 13	1256±56 12
II A		690±33 26	691±4 11	688±49 15	850±39 23	864±64 11	840±51 14	1060±54 22	1103±76 11	1018 11
II B		636±42 14	610±70 6	655±53 8	831±48 14	830±49 6	832±78 8	1051±63 11	1044±42 6	1099±138 5
III		652±60 9	685±118 3	636±45 6	828±63 9	911±145 3	787±66 6	1038±137 6	1221±230 3	856±90 3
IV		656±19 11	673±5 47	633±79 34	861±1 80	885±27 47	826±31 33	1092±27 69	1107±33 45	1065±48 24

Symbols as in Table I

A difference in working capacity might be a result of a difference in body dimensions. However, using stepwise multiple regression and partial correlation the difference remained in male types II A and IV and female type II A HLP even when the influence of body weight and age is compensated for.

In the orthostatic test there were no indications of differences in sympathetic tone between HLP and control groups. In terms of changes in HR, BP or ECG reactivity there should be only minor differences. The enormous oxygen difference between the groups during exercise (16). A difference

in stroke volume is therefore a probable explanation of the lower W_{130} value in male types II A and IV and female type II A HLP.

Therefore two main possibilities appear as an explanation for the lower W_{130} in these groups of HLP. The first is a lower degree of training. There were no significant differences in physical activity however between male control and HLP subjects as stated by the subjects in the interview, but such data are of somewhat limited value as the same training stimulus can give different effects in different individuals.

Table VI Total exercise time (min), highest work load (W_{out} , kpm/min) and the highest HR achieved (HR_{out}, beats/min)

Group	Age (y)	Men								
		All	36-50	>50	All	36-50	>50	All	36-50	>50
C			22.1	—±1	1204±51	1348±71	1047±59	162±	168±2	154.1
N			22.1	1±1	1060±46	1116±62	1012±66	158±4	167.3	140.5
II A			3.1	1±1	957±52	1125±85	834±44	158±3	170±0	149±3
II B		19	19.1	19	844±60*	953±71	763±83	151±4	160.4	145.5
III		21	4.0	70±1	925±119	1179±252	798±109	157±6	170±0	190.1
IV		1	70±1	1±1	1006±28	1088±38	842±36	159	164.2	157.5

Symbols as in Table I

no	W_{LVE}			W_{RVE}			W_{LVE}		
	36-50	>50	All	36-50	>50	All	36-50	>50	
5±19	465±33	435±43	589±22	639±37	563±27	766±28	839±47	700±3	
9	20	39	57	20	37	48	20	28	
7±22	410±38	437±27	608±33	585±47	622±46	740±38	735±82	744±18	
	11	18	29	11	18	23	9	14	
29	361±41	419±34	508±21	502±27	509±76	680±24	650±3	683±29	
	8	37	37	7	30	32	6	26	
±43	388±74	395±56	574±48	545±82	589±63	714±80	636±100	752±112	
2	4	8	12	4	8	9	3	6	
2±49	458±94	405±51	568±39	589±75	547±40	687±35	715±59	658±8	
6	3	3	6	3	3	5	3	2	
3±21	448±29	471±28	622±21	599±30	635±29	762±35	755±47	766±51	
9	9	19	26	9	17	18	7	11	

Another explanation for the smaller stroke volume might be a less effective myocardial pump function in HLP. The higher respiratory rate in the older type IIb males supports this explanation as an increase in ventilation might be an indication of depressed left ventricular function (13), especially if one considers that this higher respiratory rate was measured at 17% lower oxygen intake (work intensity). The latter explanation is supported by the finding of a lower vital capacity in the HLP groups. A decrease in vital capacity is an early sign of latent myocardial failure (18) and should not be a result of

any difference in physical activity (1). The latter explanation is further supported by the lack of correlation normally found between W_{LVE} and heart volume (19-26). This might be due to the fact that W_{LVE} was decreased disproportionately in some cases depending on a diminished left ventricular function but the lesser accuracy in heart volume measurement in standing by the hospital routine might contribute to the lack of correlation. A third factor may be a common genetic mechanism responsible for both the hyperlipoproteinaemia and changes in physiological variables, e.g. W_{LVE} and vital capacity.

total exercise time			W_{max}			HR _{max}		
J	36-50	>50	All	36-50	>50	All	36-50	>50
7±1	22±1	22±1	719±31	864±50	645±35	157±3	168±1	151±4
3±1	19±1	21±1	579±33**	544±43**	594±47	151±3	152±5**	150±4
1±1	20±1	20±1	563±28***	529±85**	570±29	150±3	158±8	149±3
1	20±1	22±1	623±44	535±44**	667±38	153±3	150±8	155±3
1	20±1	20±1	575±47	631±73	518±51	150±5	157±7	143±7
9±1	20±1	19±1	629±33	706±82	596±31	149±3	161±5	144±4

To really determine which of these explanations is valid a heart catheterization with extensive analysis of flow and pressure measurements must be performed. This has however not been possible in our subjects without clinical symptoms.

REFERENCES

- 1 Astrand P O & Rodahl K. Textbook of work physiology McGraw Hill New York 1970
- 2 Beaumont, J L, Carlson, L A, Cooper G R, Fejfar Z., Fredrickson D S & Strasser T. Classification of hyperlipidaemias and hyperlipoproteinaemias. Bull. Wild Hlth Org. 43 891 1970.
- 3 Berglund E, Birnha, G, Bjure J, Grimby G, Kjellner I, Sandqvist L & Söderholm B. Spirometric studies in normal subjects. I. Forced expiration in subjects between 7 and 70 years of age. Acta med scand. 173 185 1963
- 4 Bernstein, L., D Silva, J L. & Meadell D. The effect of the rate of breathing on the maximum breathing capacity determined with a new spirometer. Thorax 7 255 1952.
- 5 Bevergard B S & Shepherd J T. Regulation of the circulation during exercise in man. Physiol Rev 47 178, 1967
- 6 Bonanno J A. & Les, J E. Effects of physical training on coronary risk factors. Amer J Cardiol 33 760 1974
- 7 Carlson, K. Lipoprotein fractionation. J clin. Path 5 32 1973
- 8 Carlson L A. & Lindstedt, S. The Stockholm prospective study I. The initial values for plasma lipids. Acta med scand Suppl 493 1968
- 9 Ekelund L.-G. Rapid determination of work load at a heart rate of 170 beats/min with a heart-rate controlled ergometer 3 Internat. Seminar für Ergometrie, Berlin 1972.
- 10 — Heart-rate controlled ergometry Brit J Sports Med 7 121 1973
- 11 F x, S. M. Naughton, J B & Haskell W L. Physical activity and the prevention of coronary heart disease. Ann clin Res 3 404 1971
- 12 Fredrickson, D S, Levy R I & Lees, R. S. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. New Engl. J Med. 276.34 94 148 15 773 1967
- 13 Gazezopoulos, N, Davies, H, Oliver C & Denckner D. Ventilation and hemodynamics in heart disease. Brit. Heart J 28 1 1966
- 14 Grimby G & Söderholm, B. Spirometric studies in normal subjects. III. Static lung volumes and maximum voluntary ventilation in adults with a note on physical fitness. Acta med scand 173 199 1963
- 15 Hald A.. Statistical theory with engineering applications. Wiley New York 1960
- 16 Holmgren, A, Jonsson, B, Levander M, Linderholm, H, Sjöstrand T & Ström E. Low physical working capacity in suspected heart cases due to inadequate adjustment of peripheral blood flow (vasoregulatory autbenia). Acta med. scand 158 413 1957
- 17 Holmgren, A., Jonsson B & Sjöstrand T. Circulatory data in normal subjects at rest and during exercise in recumbent position, with special reference to the stroke volume at different work intensities. Acta physiol. scand. 49-343 1960.
- 18 Kennel W B., Seidman, J M, Ferbo W & Castel, B W II. Vital capacity and congestive heart failure. The Framingham study. Circulation 49-1160 1974
- 19 Kjellberg S R, Radhe U & Sjöstrand T. The relation of the cardiac volume to the weight and surface area of the body, the blood volume and the physical capacity for work. Acta radiol 31 113 1949
- 20 Olsson, A G. Studies in asymptomatic primary hyperlipidaemia. II. Clinical findings. Acta med. scand 197 477 1975
- 21 Olsson, A G & Carlsson L A. Studies in asymptomatic primary hyperlipidaemia. I. Types of hyperlipoproteinaemias and serum lipoprotein concentrations, compositions and interrelations. Acta med. scand., Suppl. 490 1. press 1975
- 22 Olsson, A G, Ekelund L.-G & Carlsson, L A. Studies in asymptomatic primary hyperlipidaemia. IV. ECG at rest and during exercise and its relation to various lipoprotein classes. Acta med scand. In press 1975
- 23 Scandinavian Committee on ECG Classification: The Minnesota code for ECG classification. Acta med scand Suppl 481 1967
- 24 Siegel, S. Non-parametric statistics for the behavioral sciences. McGraw Hill Tokyo 1956.
- 25 Sjöstrand T. Experimental variations in the T wave of the electrocardiogram. Acta med scand 138 131 1950
- 26 — Functional capacity and exercise tolerance in patients with impaired cardiovascular functions. Clinical cardiopulmonary physiology. Grune & Stratton, New York 1960
- 27 Snedecor G W & Cochran W G. Statistical methods. Iow. State College Press. Iowa 1967
- 28 Wood F D, Klem H, Lewis S & Haskell, W. Plasma lipoprotein concentrations in middle-aged male runners. Circulation Suppl III 115 1974

ANTIHYPERTENSIVE EFFECT OF β -1 RECEPTOR BLOCKADE AND β -2 RECEPTOR STIMULATION IN ESSENTIAL HYPERTENSION

Owe Andersson and Göran Berglund

From Medical Department A I H hypertension Clinic Sahlgrenska Hospital
University of Göteborg Göteborg Sweden

Abstract The antihypertensive effect of practolol β -1-blocker alone and in combination with β -2-stimulating drug, salbutamol has been studied in double-blind cross-over trial on 19 patients. Practolol treatment induced significant BP reduction while the addition of salbutamol failed to give any further decrease.

Animal studies have indicated that β -receptors can be divided into two groups. Stimulation of the β -1-receptors causes a increase in heart rate (HR) and cardiac output (\dot{Q}) and lipolysis while stimulation of β -2-receptors gives bronchial smooth muscle relaxation and peripheral vasodilatation (8-9).

Practolol a β -1-receptor blocker has been shown to have the same BP-lowering effect as propranolol (6). Salbutamol a β -2-receptor stimulant, has a vasodilating effect and its duration on oral administration is \sim 3 times longer than that of isoprenaline (5,4). Theoretically a therapy which combines these two agents would be expected to have a greater antihypertensive effect than practolol alone because peripheral resistance would be reduced by the vasodilating effect of salbutamol. The reflex baro-receptor mediated increase in HR and \dot{Q} would be blocked by practolol.

The aim of the present study was to determine whether the combined administration of practolol and salbutamol gave a better BP reduction than practolol alone.

MATERIAL AND METHODS

Twenty previously untreated hypertensive patients with an average age of 41 years (range 40-46), 18 men and

two women, entered the trial. All patients were recruited from an ongoing population study (1) and had casual BP higher than 175 and/or 115 mmHg on two consecutive readings. They were then followed until the BP stabilized after 3-6 out-patient controls. All had benign essential hypertension. The female patients ($n=2$) did not use oral contraceptives. One patient was excluded because of tablet failure, i.e. intake of less than 90% of the prescribed number.

All patients were examined and followed at the Hypertension Clinic, Sahlgrenska Hospital. The examination included ECG and X-ray of the chest, analyses of albumin and glucose, serum creatinine, uric acid and serum electrolytes. Eye-ground examination was done by the investigators. BP was measured in the right arm, the subject and standing position after 5 min rest with mercury manometer (to the nearest mmHg). Diastolic BP-phase 5 was used. HR was determined by pulse palpation. With this examination 11 subjects were classified as WHO stage I while 8 showed signs of left ventricular hypertrophy or eye-ground changes as in WHO stage II.

Design of the study

All subjects were treated during a run-in period with practolol, 700 mg three times daily for one month. Thereafter salbutamol 4 mg three times daily or placebo was added for one month each in double-blind cross-over trial. BP and HR were measured at the end of each period.

RESULTS

Table I shows the mean BP and HR for the whole group before and after treatment during one month. On practolol systolic and diastolic BP as well as HR were significantly lower than before treatment ($p<0.05$). Table I also shows the average BP and HR after addition of salbutamol and placebo. No further BP reduction was achieved with the addition of salbutamol. No orthostatic hypotensive reactions or side-effect e.g. bradycardia, gastrointestinal symptoms or tremor were noted.

Address for reprints: O. Andersson, Medical Department A Sahlgrenska Hospital S-413 45 Göteborg, Sweden.

Table 1 Mean blood pressure and heart rate in 19 patients before treatment and during the three treatment periods

	Before treatment			On practolol			On practolol and placebo			On practolol and salbutamol		
	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR
1	172	106	79	163	101	67	159	98	67	160	97	77
	16.7	7.6	11	18.3	9.9	9.7	18.4	10.0	7.3	19.9	11	11.1

DISCUSSION

The results from the present study are not encouraging for the use of this drug combination in the general treatment of hypertension. It is not clear why no further BP reduction was achieved by the addition of salbutamol to the previous practolol treatment. The present oral doses of salbutamol have been shown to be adequate to give β_2 -receptor stimulation in the bronchial smooth muscles. Larger doses of salbutamol also carry a greater risk of tremor (3, 7).

Possible explanation of the lack of BP reduction on salbutamol might be structural or functional differences between the β_2 -receptors in the bronchial and in the peripheral arteries. The data do not show whether Q increased when salbutamol was added but the slight (although not significant) increase in HR may indicate that this was the case.

Treatment with β_1 -receptor blockers and β_2 -receptor stimulant seems to be a useful combination in hypertensive patients with obstructive lung disease (1). Further studies are needed to determine whether larger doses of salbutamol would give BP reduction in combination with β_1 -receptor blockade.

REFERENCES

- Andersson, O. & Berglund, G. Practolol—propranolol. A comparison of antihypertensive effect. *Acta med scand* 196; 479, 1974.
- Cullum, V. A., Farmer, J. M., Jack, D. & Levy, G. P. Salbutamol: a new selective beta-adrenoceptor receptor stimulant. *Brit J Pharmacol* 35; 141, 1969.
- Epikelet, S. W., Barnard, J. A. & Zoller, T. T. A clinical trial of long-term oral salbutamol in reversible diffuse airway obstruction. *Amer Rev resp Dis* 108; 1973.
- Forngren, H. Practolol in the treatment of tachyarrhythmias in patients with bronchial asthma. *Amer Heart J* 84; 710, 1972.
- Furchgott, R. F. Pharmacological characteristics of adrenergic receptors. *Fed Proc* 29; No 4, 1970.
- Lands, A. M., Arnold, A., McAuliffe, J. P., Laddara, F. P. & Brown, T. G. Jr. Differentiation of receptor systems activated by sympathomimetic amines. *Nature* 14; 997, 1967.
- Marden, C. D. & Meadows, J. A. The effect of adrenaline on the contraction of human muscle. *J Physiol (Lond)* 194; 70, 1966.
- Phillips, E. M., Woolnough, M., Marinova, V. M. & Turner, P. A comparison of isoprenaline, salbutamol and ritoterol inhalation on skin temperature, heart rate and respiration in man. *clin Pharmacol* 138; 1972.
- Willemsen, L. R. & Werk, J. Prevalence and distribution sample. *Acta med scand* 2; 47, 1973.

A POSTMYOCARDIAL INFARCTION CLINIC IN GÖTEBORG SWEDEN

A Follow-up of MI Patients in a Specialized Out-patient Clinic

Dag Elmfeldt, Lars Wilhelmsson Gösta Tibblin, J Anders Vedin,
Claes-E Wilhelmsson and Calle Bengtsson

*From the Department of Medicine Section of Prevalence in Cardiology Sahlgren Hospital
University of Göteborg Göteborg Sweden*

Abstract. The registration of all myocardial infarction (MI) in the city of Göteborg started on Jan. 1st 1968 when a special clinic was set up for ambulatory posthospital care of infarction patients. In 1970 this clinic was expanded to cover all patients below 67 years of age with MI in the city of Göteborg, the aim being to standardize and unify patient care and therapeutic regimens, to provide opportunities for the study of patient characteristics, natural history, risk factors and effects of preventive measures. Results from such studies have been published, but so far no detailed description of this special out-patient unit, nor of any similar unit elsewhere. Patient recruitment, considerations concerning personnel, patient education procedures and return visit routines are covered, together with investigative methods and criteria for the treatment of complications, symptoms and risk factors. The cumulative drop-out rate up to and including 2 years follow-up was only 3.5%. A brief bibliography of studies originating at the Postmyocardial Infarction Clinic is included.

Patients who survive myocardial infarction (MI) run a great risk of reinfarction. Previous MI may be said to be the dominant risk factor for recurrence (25, 30, 31, 33). There is a great risk of sudden death (29) and in many survivors there is a considerable degree of invalidity (8, 32, 38, 39). It would therefore be natural to subject postinfarction patients to systematic follow-up and thorough care. This has been done but only within selected groups of patients and for certain specific purposes, such as the study of the prognosis or secondary prevention by

means of for example anticoagulants or diet (1, 6, 14, 18, 22).

A retrospective investigation of men below 50 years of age who survived their first MI which had occurred between 1948 and 1965 in Göteborg showed that their medical care with respect to known risk factors such as high BP, smoking and hypercholesterolemia, had been very heterogeneous. One-third of the patients were not under medical supervision at all (13).

In Göteborg there is only one hospital for the acute care of MI patients which is one reason why there are good possibilities of registering all cases of acute myocardial infarction (AMI) for the purpose of epidemiological studies (9, 10, 77). Against this background it seemed logical to start an ambulatory clinic for uniform follow-up of postinfarction patients—the Postmyocardial Infarction Clinic (9, 76, 27, 34). The establishment of a register for all cases of infarction enabled 90% of all diagnosed surviving cases to be registered though with certain age limitations owing to practical problems of capacity (9, 10).

The purpose of the Postmyocardial Infarction Clinic was to enable in a representative infarction material 1) regular standardized ambulatory control and treatment after acute care, 2) comparison of data from patients who have suffered MI with data from the total population in order to characterize the infarction patient, 3) study of the course after MI, 4) identification of factors of importance for the course—secondary risk factors.

Address for reprints: D Elmfeldt, MD, Department of Medicine Sahlgren Hospital S-41345 Göteborg, Sweden.

development of secondary preventive measures (prevention of e.g. invalidity, reinfarction and death).

PATIENTS

The patients were identified while undergoing acute care through the previously mentioned register of all infarction patients. During 1968-69 men born in 1913 or later who had survived an AMI after Jan. 1st 1968 were cared for at the Postmyocardial Infarction Clinic. During 1970 and 1971 men from the population of Göteborg born in 1903 or later were cared for with the exception of those born between 1903 and 1911 on the 3rd, 6th, 9th, 13th, 16th, 19th, 23rd, 26th or 29th of the month (e.g. a random 30% sample) who were excluded in order to form a reference group. The latter patients were given the previous conventional treatment. At present patients born in 1914 or later are cared for.

STAFF OF THE CLINIC

The medical duties have been shared by six physicians, all of whom serve at the Clinic on a part-time basis. The total medical service has gradually increased and is estimated to 60 h/week for 1974. The nursing service has increased continuously and amounted in 1974 to 40 h/per week for a state-registered nurse and 80 h/week for two assistant nurses. Secretarial duties amounted in the same year to 40 h/week.

PATIENT INFORMATION

During 1968-71 the patients were contacted while receiving acute care by one of the physicians from the Postmyocardial Infarction Clinic. The first control examination at the Clinic was held 1-3 weeks after discharge from hospital.

When first contacted by the physician from the Clinic the patient was given information concerning his disease and the necessity of regular check-ups after discharge. The Postmyocardial Infarction Clinic and its activities were described and the patient was offered regular follow-up and control. The information included an explanation of what is meant by arteriosclerosis in general and particularly MI. The risk factor concept was also discussed. In connection with the discussion concerning risk factors, smokers were encouraged to stop smoking. The connection between saturated fats and serum cholesterol was explained and the patients were encouraged to reduce their intake of saturated fats. Individual instructions concerning the suitable level of activity during convalescence were given to each patient. In general a gradual

increase in activity in the form of walks was advised commencing with 5-10 min at a gentle pace and avoiding hills and steps. It was suggested that the number and duration of walks be increased from day to day eventually including hills and broken ground. The patients were advised to refrain from sexual activity during the first four weeks. After an individually adjusted period they were informed that all activity was permitted and indeed desirable as long as it did not cause chest pain, pronounced dyspnoea or tiredness during or shortly after the activity in question.

At the first appointment the principles for determining when and whether return to work was possible were explained. The patient's occupation and age and the extent of the infarction including complications were taken into consideration in this respect. Subsequent discussions concerning return to work were based on these data as well as on symptomatology and the extent of invalidity.

In addition to oral information the patients were given printed information in the form of special brochures developed at the Postmyocardial Infarction Clinic. Three publications dealing with MI (36), diet (37) and smoking (35) have been issued. In addition information was given partly by means of audiovisual aids to groups of 10-15 patients together with members of their families. This group information took place 3-4 weeks after discharge. As from 1972 the first contact between the patients and the Postmyocardial Infarction Clinic is established prior to discharge from hospital by a nurse giving information and delivering the printed information material.

ROUTINES FOR CHECK UP VISITS

Check-up examinations by the physicians at the Postmyocardial Infarction Clinic took place at intervals indicated by the clinical situation. In addition control examinations were always performed at fixed times according to a predetermined schedule. During 1968-71 these control examinations were performed for patients born in 1913 or later at 3, 12, 4 and 60 months after the infarction. During 1970-71 control examinations were performed for patients born between 1903 and 1912 after 1 and 3 weeks and after 3, 6, 12, 18 and 4 months and annually thereafter. For this group of patients check-up examinations by nurses were tested during these years as a complement to

check-ups by physicians. A specially trained nurse examined the patient every 6 weeks up to and including 24 months after the infarction. She took a standardized case history concerning the period since the previous examination with the emphasis on chest pain, decompensation and arrhythmias. She also recorded the BP, pulse and weight and performed laboratory tests for Hb, ESR, serum potassium and glucosuria or proteinuria. In the event of any of the following she contacted the physician responsible for the patient: increased angina pectoris, suspected reinfarction and/or decompensation, diastolic BP ≥ 105 mmHg, change of weight, or newly developed irregularity of pulse. The physician was also contacted if serum potassium was ≤ 3.6 mEq/l and in the event of anemia, ESR rise or glucosuria. In addition, the responsible physician was contacted in any situation in which the nurse was in doubt in any way.

The organization, experience and certain results for 1968-71 have partly been reported previously (4, 8, 9, 13, 16, 28, 30, 34, 38). In the light of experience, the present routine for check-ups was developed with fixed controls by physicians after 4, 12, 30 and 52 weeks and after 2 and 5 years, and controls by nurses after 8, 20 and 40 weeks.

INVESTIGATIONS

Blood pressure. BP was determined, in accordance with WHO's recommendations (22) at each check-up visit in the right arm using a mercury manometer recording to within 2 mm, supine and standing and after rising. Primarily the pressure was rapidly pumped up to 200 mmHg. Systolic BP was recorded when the Korotkoff sounds occurred, diastolic when the Korotkoff sounds disappeared (phase 5).

Heart rate. From the ECG tracing (see below) 5 R-R intervals were determined. The value obtained was converted nomographically to HR. In the event of very irregular rhythm, e.g. atrial fibrillation, the number of beats/30 cm was counted. HR was recorded at each control visit.

Electrocardiogram. Leads I, II, III and AVR, AVL, AVF and CR. CR, CR₁, CR₂ and CR were registered routinely at each control visit after 5 min rest. The apparatus used was an Elema Schöander type Mingograf 34, paper speed 50 mm/sec. Exercise ECGs on a bicycle ergometer with stepwise increases of the load were taken at the 3, 12 and

24-month controls for patients born in 19 (24).

Heart-lung X-ray. X-ray of the heart was performed routinely in the standing position at 0, 3, 6 and 12 months after infarction. Patients who had suffered infarction between 1968 and 1971 were patients suffering infarction in 1971 months after the infarction. Patients who had suffered infarction in 1977 or later were not X-rayed after discharge, since experience had from a clinical point of view there was no need to perform this investigation routinely. Total relative cardiac volumes were calculated to Jonzell (15).

Lipids and lipoprotein electrophoresis. Cholesterol and serum triglyceride determined and typing of lipoproteins electrophoresis was performed at 3, 6 and 12 months after infarction. As from March 1970 these were also performed 6 months after the acute infarction. The blood samples were taken in the morning after 12 hours fasting. Serum cholesterol was determined according to Cramer and Isaksson (7) and serum triglycerides according to Carlén (8). Serum lipoprotein electrophoresis was performed on filter paper mainly according to the method described by Lees and Hatch (17) and on agarose gel mainly according to Rapp and Kahle (21). The serum lipoprotein patterns were typed biochemically according to Fredrickson and Lees (11) and Beaumont et al. (2). A detailed description of the lipid and lipoprotein determinations has been presented previously (12).

Routine laboratory tests. At each control Hb and ESR were recorded and a urine sample was taken for albuminuria (Albumix®) and glycosuria (Clinistix®). The patients were also weighed. As from 1972, serum potassium was determined at each check-up.

Special investigations. Coagulation factors and fibrinolysis were studied in connection with the 3-month and 12-month controls in the groups of patients who suffered infarction in 1968 and 1969. The methods have been described in detail previously (16). Body composition was determined using K-isotope technique 3 and 12 months after the infarction in patients who suffered infarction in 1968 (19). Serum electrophoresis, fibrinogen, ANF, liver status, serum creatinine and serum potassium were checked at the 6 and 12-month controls in patients born between 1903 and 1912 in the 1970 material.

Dietary habits were determined by interview by the same dietitian at 3 and 4 months after the infarction in the 1968-69 groups.

COMPUTER REGISTRATION

For uniform and complete registration of data, special case history forms were prepared for direct transfer of information to punch cards for computer processing (9). These computer case histories comprised personal statistics, family history and social circumstances. Information on certain previous diseases, cardiovascular and pulmonary symptomatology, data concerning smoking habits and physical activity at work and during leisure hours and information concerning subjective stress. Conditions at the time of infarction, during the period of acute care and at discharge, as well as during follow-up, were also registered. Details of the case history were obtained with the same questionnaire as was used in the population studies of men and women in Göteborg, and which is partly based on International questionnaires recommended by the WHO (7).

TREATMENT PRINCIPLES

The Postmyocardial Infarction Clinic treated patients exclusively on an ambulatory basis, which meant that medication at discharge was not influenced. The Clinic subsequently applied fixed criteria for the treatment of symptoms, complications and risk factors. The treatment principles were continually discussed within the group, so that the care of the patients was uniform both as regards criteria for indications and therapy.

Angina pectoris. Effort-induced pain which was clinically assessed to be caused by myocardial ischemia was treated with sublingual nitroglycerine. Patients with severe and/or frequent attacks were treated with β -blockers as well.

Decompensation. Patients with dyspnoea on exertion, increase of weight, oedema of the legs and/or venous stasis, with or without X-ray signs of heart failure, were given digitalis in the first place. In cases in whom this was considered to be insufficient, saluretic diuretics and potassium supplements were also given, and if this was inadequate, aldosterone antagonists or a similar drug was also given.

Arrhythmia. In the event of arrhythmia, the adequacy of treatment for decompensation, the pres-

ence or absence of hypokalemia and the possibility of the arrhythmia being drug-induced were considered. Conventional antiarrhythmic treatment was given. Ventricular extrasystoles were not treated unless special indications were present (e.g. paired ectopic beats during effort).

Hypertension. Patients below 55 years with a systolic BP ≥ 160 mmHg and/or diastolic BP ≥ 105 mmHg, and patients above 55 years with a diastolic BP ≥ 105 mmHg on two subsequent occasions were given antihypertensive treatment. When pressures at these levels were first recorded, the patient was booked for a check-up within one month. For moderate hypertension during 1968-69, a saluretic diuretic with potassium supplements was used in the first place if the serum uric acid level was normal. Alternatively, α -methyldopa and betanidine, possibly combined with hydralazine, were used. As from 1970, a saluretic diuretic or a β -blocker was used as first-line treatment. If control of the BP was not adequate, hydralazine was added to the basic drug. In certain severe cases, more complicated combinations were used. The objective of treatment for patients below 55 years was a BP $\leq 150/100$ mmHg, and for older patients a diastolic BP ≤ 100 mmHg.

Hyperlipidemia. Cholesterol ≥ 300 mg/100 ml and/or triglycerides ≥ 175 mg/100 ml were treated by dietary measures. In certain cases, and after special clinical assessment, clofibrate and/or nicotinic acid were also given.

Smoking. At the time of the first contact, while the patient was receiving acute care, oral and written anti-smoking counselling was given. The importance of strict abstinence from smoking was also emphasized during the follow-up. A sedative was given if necessary to help the patient stop smoking.

Anticoagulants. Patients with enlargement of the heart and concurrent atrial fibrillation (either stationary or recurrent) and/or left ventricular aneurysm and/or signs of extensive arteriosclerotic disease, for example intermittent claudication prior to infarction, were given long-term treatment with dicoumarol.

As regards other diseases and symptoms, the principle was to care for the patient at the Clinic to the greatest possible extent, either directly or indirectly via referral to a suitable specialist. The management of patients on prolonged sick-leave was discussed at regular meetings between the physicians and nurses from the Postmyocardial Infarc-

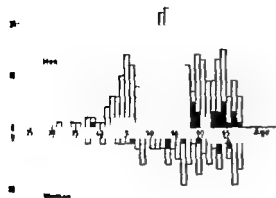


Fig. 1 Patients cared for at the Postmyocardial Infarction Clinic during 1968-70 by age and sex. ■ = patients who came under care due to reinfarction. ▨ = 36-year-old comprise only patients who had their infarct during 1968-70. 57-67-year-olds comprise only patients from 1970.

tion Clinic the physicians from the Rehabilitation Clinic and the medical advisors and staff of the Göteborg Regional Social Insurance Office.

PARTICIPATION RATE AND SUMMARY OF THE NATURAL HISTORY

During 1968-70 479 patients (391 men, 88 women) were cared for at the Postmyocardial Infarction Clinic. These patients included 64 men and 16 women who had previously suffered an MI and who thus came under the care of the Clinic due to reinfarction (Fig. 1). Few patients failed to accept the offer of controls at the Clinic. At the first visit four of the 479 patients did not attend. The cumulative number of absentees up to and including 2 years follow-up was 13 of 414 survivors, i.e. 3%.

One year after infarction 56% of the survivors were back at work, 26% were on sick-leave and 18% were receiving disability pensions. For those who had returned to work the median duration of sick-leave was 19 weeks. At the control 1 year after infarction 47% of the patients were receiving diuretics and 19% were taking a diuretic.

Certain aspects of the infarction studies which began in 1968 have been published. The characteristics of young female infarction patients were presented in a thesis in 1973 (3). In the same

year studies on exercise tolerance and ability to undergo physical training after MI were presented in another thesis (74). The characteristics of infarction patients compared with the general population (9), the natural history during the acute phase and during follow-up (38) and prognostic aspects (30) have been presented in three other theses.

REFERENCES

1. Aspenström G & Korsan-Bengtsson, K. A double blind study of dicumarol prophylaxis in coronary heart disease. *Acta med. scand.* 176: 563 1964.
2. Beaumont, J. L., Carlson, L. A., Cooper III R., Fajar Z., Fredrickson, D. S. & Strasser T. Classification of hyperlipoproteinemias and hyperlipidemia. *Bull. WHO High Org.* 43: 891 1970.
3. Bengtsson, C. Ischaemic heart disease in women. *Acta med. scand. Suppl.* 549 1973.
4. Bengtsson, C., Blomgren H., Hallberg, L., Hållström, T., Isaksson, B., Korsan-Bengtsson, K., Rybo, G., Tibblin, E., Tibblin, G. & Westerberg, H. The study of women in Göteborg 1968-1969—a population study. General design, purpose and sampling results. *Acta med. scand.* 193: 311 1973.
5. Carlson, L. A. Determination of serum triglycerides. *Acta Soc. Med. Scand.* 64: 208 1959.
6. Coronary Drug Project Research Group: *Amer. Heart Ass. Monograph No. 38*, 1973.
7. Croner K. & Isaksson, B. An evaluation of the Thorén method for the determination of total serum cholesterol. *Scand. J. clin. Lab. Invest.* 11: 13 1959.
8. Elmfeldt, D. & Wilhelmsson, L. A study of representative postmyocardial infarction patients aged 72-85. In: *Preventive cardiology* (ed. G. Tibblin, A. Kuylenstierna & L. Wilhelmsen) pp. 129-139. Almqvist & Wiksell, Stockholm 1972.
9. Elmfeldt, D. Hjärtinfarkt Göteborg 1968-1970. Morbiditet och mortalitet. Karakteristika för överlevande och döda. Jämställning med den manliga befolkningen. *Ekstrand, Kongsholm* 1974.
10. Elmfeldt, D., Wilhelmsson, L., Tibblin, G., Vedin, J. A., Wilhelmsson, C. E. & Bengtsson, C. Registration of myocardial infarction in the city of Göteborg, Sweden—A community study. *J. chron. Dis.* In press 1975.
11. Fredrickson, D. S. & Lees, R. S. A system for phenotyping hyperlipoproteinemia. *Circulation* 31: 321 1966.
12. Gustafsson, A., Elmfeldt, D., Wilhelmsson, L. & Tibblin, G. Serum lipids and lipoproteins in men after myocardial infarction compared with representative population sample. *Circulation* 46: 709 1972.
13. Hood B., Tibblin, G., Wehn, G., Örn Dahl, G. & Korsan-Bengtsson, K. Myocardial infarction in early age III. Coronary risk factors and their deficient control. *Acta med. scand.* 183: 41 1969.
14. International Anticoagulant Review Group: Collaborative analysis of long-term anticoagulant

- administration after acute myocardial infarction. *Lancet* 1: 203 1970.
- 15 Jonell S.. A method for determination of the heart size by teleroentgenography (a heart volume index). *Acta radiol* 20: 325 1939
 - 16 Korsan-Bengtson L, Wilhelmson L, Elmfeldt D & Tibblin G. Blood coagulation and fibrinolysis in men after myocardial infarction compared with a representative population sample. *Atherosclerosis* 16: 83 1972.
 - 17 Lees R S & Hatch, F T. Sharper separation of lipoprotein species by paper electrophoresis in albumin containing buffer. *J Lab. clin Med.* 61: 518, 1963
 - 18 Leren P. The Oslo diet-heart study—eleven year report. *Circulation* 42: 935 1970
 - 19 Ljungholm B. Some aspects of body composition in patients with myocardial infarction. *Pekr Dubb J* 3: 55 1969
 - 20 Lovell R. R. H. Rethinking coronary thrombosis: lessons from experimental therapeutics with anticoagulants. *Med J A st* 425 1969
 - 21 Rapp W & Kahle W. Lipoprotein-electrophoresis in agarose gel. *Clin. chim. Acta* 19: 493 1968
 - 22 Rose G & Blackburn, H. Cardiovascular survey methods. WHO Geneva 1968
 - 23 Sæne H, Elmfeldt, D & Wilhelmson L. Preventive effect of physical training after a myocardial infarction. In: *Preventive cardiology* (ed O Tibblin, A. Keys & L. Werkö) pp 154-160 Almqvist & Wiksell Stockholm 1972.
 - 24 Sæne H. Exercise tolerance and physical training of non-selected patients after myocardial infarction. *Acta med scand Suppl* 551 1973
 - 25 Slevens, J. Myocardial infarction. *Acta med scand Suppl* 406 1963
 - 26 Tibblin, G. *Preventiv kardiologi*. Göteborg, Läkarsällningen 66 5:39 1969
 - 27 Tibblin G. Rehabilitering av hjärtinfarktpatienter ef III sjukhus stegen—infarkt-mottagningen. *Läkarsällningen* 66 2413 1969
 - 28 Vedin, A & Wilhelmsson, C. E. Evaluation of a myocardial infarction out-patient clinic—A secondary preventive trial. In: *Preventive cardiology* (ed G. Tibblin, A. Keys & L. Werkö), pp. 161-165 Almqvist & Wiksell Stockholm 1972.
 - 29 Vedin, J. A., Wilhelmsson, C. E., Elmfeldt, D, Tibblin G, Wilhelmson, L. & Werkö, L. Sudden death: Identification of high risk groups. *Amer Heart J* 86: 124 1973
 - 30 Vedin J. A., Hjärtinfarkt i Göteborg 1968-1970. Dödsfall, infarktscidi och prognosfaktorer under två års uppföljning av patienter som överlevt akut koronarsjukdom. *Elanders, Kungälv* 1974
 - 31 Vedin, J. A., Wilhelmson, L., Wedel H, Persson, B., Wilhelmsson C. E., Elmfeldt, D & Tibblin, G. Multivariate assessment of risk of death and reinfarction after myocardial infarction. To be published.
 - 32 Weinblatt, E., Shapiro S., Frank, C. W & Sager R. V.. Return to work and work status following first myocardial infarction. *A.J.P.H* 36: 149 1966
 - 33 — Prognosis in man after first myocardial infarction—mortality and first recurrence in relation to selected parameters. *A.J.P.H* 38: 1329 1968.
 - 34 Wilhelmson, L. The myocardial infarction clinic in Göteborg—organization and preliminary results. *Pekr Dubb J* 3: 43 1969
 - 35 — Om Du ska bli slott röka. *Nationalföreningen för upplysning om tobakens skadeverkningar* Stockholm 1971
 - 36 Wilhelmson L. & Werkö L. Hjärtinfarkt. *Svenska Nationalföreningen mot hjärt och kärlsjukdomar* Stockholm 1971 1974
 - 37 Wilhelmson L., Björk, T & Björkman A.-C. Blodfett, fetma och åderförkalkning. *Scandmed, Göteborg* 1973
 - 38 Wilhelmsson, C. E. Hjärtinfarkt i Göteborg 1968-1970. Analys av fynd under sjukhusvistelse och efterförlopp. *Elanders, Kungälv* 1974
 - 39 Wilhelmson, C. E., Elmfeldt D, Vedin, J. A., Tibblin G & Wilhelmson, L. Functional impairment after myocardial infarction. To be published

CREATINE PHOSPHOKINASE AFTER SUBMAXIMAL PHYSICAL EXERCISE IN UNTRAINED INDIVIDUALS

G Forssell, R. Nordlander O Nyquist
E. Orinius and I Styrellus

*From the Department of Medicine Krokusala Institute
at Huddinge Hospital Huddinge S edm*

Abstract Serial estimations of total serum creatine phosphokinase (CPK) have been performed before and during 18-49 hours after submaximal physical exercise in 17 untrained individuals, mean age 40 years. The maximal CPK increase after exercise was 12 mU/ml (75%). The serum CPK did not exceed the upper normal limit (130 mU/ml) except in one individual (150 mU/ml). The maximal CPK increase in patients with acute myocardial infarction (AMI) varied between 101 mU/ml (133%) and 2 260 mU/ml (3 790%), mean 900 mU/ml (1 184%). As the maximal CPK elevation in AMI occurs within the same period it seems that heavy physical work of short duration just before the onset of symptoms will very seldom impair the diagnosis of AMI with the CPK technique and

Serial estimations of serum creatine phosphokinase (CPK) have gained much importance in the diagnosis of acute myocardial infarction (AMI). As CPK rises earlier above normal level and reaches its maximum earlier than other commonly used enzymes it provides a very valuable test in the early phase of AMI (3 11 15 19). CPK has been documented to have a very high sensitivity in AMI and the CPK range is about double the GOT range in the same patients. However there has been much argument about the specificity. Significant CPK elevations have been reported after e.g. physical exercise (1 9 11 14, 18) in injections of various drugs (?) cardioversion of arrhythmias (6) and in muscular diseases (5). On the other hand, these studies have been performed with rather infrequent blood sampling, mostly only once a day.

The aim of the present study was to investigate the change in total CPK after submaximal physical exercise in untrained individuals of different ages by frequent blood sampling.

MATERIAL AND METHODS

Seventeen untrained subjects, 15 men and 2 women, performed a stepwise increasing exercise on a bicycle ergometer. Their ages ranged from 32 to 73 years (mean 50). The starting load for the men was 50 W with increments of 30 W every 6 min. The women started on 40 W with increments of 30 W every 6 min as far as possible. None had any history of angina pectoris or myocardial infarction and none experienced chest pain during their exercise test. No i.v. injections were given before or after the test.

Venous blood samples for total serum CPK analysis were taken 30 min before, just prior to the bicycle test, immediately after 30 min after 1 hour after and every 2nd-4th hour for 18-49 hours. The blood sample was centrifuged and the serum was frozen to -18°C for 1-3 days. All blood samples from one individual were analysed together using the same CPK reagent for the whole batch. The CPK activity was measured at 37°C with spectrophotometer (LKB Reaction Rate Analyzer 9600) using a test pack (Boehringer® 157/1). The CPK activity is expressed in mU/ml (Enzyme nomenclature Recommendations 1964 of the International Union of Biochemistry Elsevier Amsterdam, London and New York 1965).

The maximal difference when analysing double samples varied from 0 to 56 mU/ml for CPK values between 16 and 2 186 mU/ml. Comparison of the CPK activity in samples analysed immediately and after freezing for 1-41 days (mean 8) showed a difference varying from 0 to 42 mU/ml, for CPK values between 21 and 1 125 mU/ml. These differences are not statistically significant ($p > 0.05$).

RESULTS

The 17 individuals stopped their exercise test on 40-300 W (mean 130±67) because of muscular fatigue. None experienced any chest pain. The CPK values are shown in Fig. 1. The maximal CPK elevation immediately after the exercise test was 1

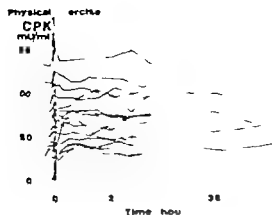


Fig. 1 CPK following physical exercise (indicated by arrow)

mU/ml (18%). The maximal CPK increase during the following 18–49-hour period was 3 mU/ml (73%). In no subject did CPK rise above 150 mU/ml after exercise, and only one individual had a CPK value above the upper normal level (130 mU/ml with our technique).

In 97 consecutive AMI patients the maximal CPK increase varied between 101 (133%) and 760 mU/ml (3790%), mean 900 mU/ml (1184%).

DISCUSSION

The effect on serum CPK activity of various forms of physical exercise has been variously reported by different authors (1, 8–14, 16, 18, 20). The conflicting results may be due to differences in age and physical state of the subjects, the nature and duration of the exercise, the intervals of blood sampling, the duration of the follow-up after exercise and different methods for CPK analysis.

Most of the studies mentioned concern young healthy students, trained or untrained, and young athletes. In the present study the mean age (50 years) was not as far from that of patients with AMI and only minimal CPK elevations were observed in each individual. Minimal CPK elevations are uncommon in AMI diagnosed by history, ECG and serum GOT/GPT. Heavy physical exercise just before the onset of symptoms is also uncommon. So diagnosing AMI with the aid of serial CPK determinations will very seldom be impaired by heavy physical work of short duration prior to the admission.

Assuming that the observed CPK elevations after exercise are due to the skeletal muscle release, it

should be possible for diagnostic purposes to differentiate this source from the myocardium by using the CPK isoenzymes. There are, however, divergent opinions about the proportion of CPK MB in skeletal muscle: 0–20% (4, 17, 19) as well as about the proportion of CPK MB as a percentage of total CPK in association with AMI: 0–38% (7, 19). There is accordingly a certain risk of a false positive as well as a false negative diagnosis of AMI even with the aid of CPK MB isoenzymes.

In conclusion, only insignificant elevations of total CPK occurred during 18–49 hours after submaximal physical exercise. Heavy exercise performed within this period will therefore apparently only seldom interfere with the diagnosis of AMI by serial estimation of total CPK.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Swedish National Association against Heart and Chest Diseases and was given technical support by LKB-produkter AB, Sweden.

REFERENCES

1. Ahlborg B & Brodusht J. Metabolic changes after exercise. *Lancet* 1: 1272, 1966.
2. Casace L. Elevated serum CPK after drug injections. *New Engl. J. Med.* 287: 309, 1972.
3. Goodley E. Prognostic value of enzymes in myocardial infarction. *J.A.M.A.* 225: 597, 1973.
4. Dawson D & Fine J. Creatine kinase in human tissues. *Arch. Neurol.* 16: 175, 1967.
5. Hughes B P. Creatine phosphokinase in facioscapulohumeral muscular dystrophy. *Brit. med. J.* 3: 464, 1971.
6. Kottinen A, Hopff V., Louhija A & Härtel G. Origin of elevated serum enzyme activities after direct-current counter-shock. *New Engl. J. Med.* 281: 231, 1969.
7. Kottinen A & Somer H. Determination of serum creatine kinase isoenzymes in myocardial infarction. *Amer. J. Cardiol.* 29: 817, 1972.
8. Ledwith J. Changes in serum creatine phosphokinase during submaximal exercise testing. *Canad. med. Ass. J.* 109: 773, 1973.
9. Loegering D, Critz J & Wagner J. Serum creatine phosphokinase as a diagnostic aid. *Miss. Med.* 50: 1751, 1967.
10. Martin K. J. & Nichols O. Serum creatine phosphokinase in man during diving training. *Aerospace Med.* 45: 67, 1974.
11. Nevins M A, Saran M, Bright M & Lyon L. J. Pitfalls in interpreting serum creatine phosphokinase activity. *J.A.M.A.* 224: 1382, 1973.
12. Nutall F & Jones B. Creatine kinase and glutamic oxalacetic transaminase activity in serum. Kinetics of

- change with exercise and effect of physical conditioning. *J. Lab. clin. Med.* 71: 847, 1968.
11. Pearce, J., Pennington, R. & Walton, J. Serum enzyme studies in muscle disease. *J. Neurol. Neurosurg. Psychiat.* 27: 1, 1964.
 14. Shapiro, Y., Magazaniuk, A., Sokar, E. & Reich, C. Serum enzyme changes in untrained subjects following prolonged march. *Canad. J. Physiol. Pharmacol.* 51: 771, 1973.
 15. Sobel, B. E. & Shell, W. E. Serum enzyme determinations in the diagnosis and assessment of myocardial infarction. *Circulation* 45: 471, 1972.
 16. Swinnea, K. D. & Awad, E. A. Creatine phosphokinase and other enzyme activity after controlled exercise. *Neurology* 14: 977, 1964.
 17. van der Veen, K. J. & Willebrands, A. F. Isoenzymes of creatine phosphokinase: tissue extracts and in normal and pathological sera. *Clin. chim. Acta* 13: 314, 1966.
 18. Veijilä, A. & Teasdale, G. Serum creatine kinase and physical exercise. *Brit. med. J.* 1: 1653, 1965.
 19. Wagner, G. S., Roe, C. R., Lumbard, L. E., Rosati, R. A. & Wallace, A. G. The importance of identification of the myocardial-specific isoenzyme of creatine phosphokinase (MB form) in the diagnosis of acute myocardial infarction. *Circulation* 47: 263, 1973.
 20. Wolfson, S., Rose, L., Boerser, J., Parra, A., Acosta, A., Cooper, K. & Schechter, E. Serum enzyme levels during exercise in patients with coronary heart disease: Effects of training. *Amer. Heart J.* 84: 478, 1972.

GAUCHER'S DISEASE AND BENIGN MONOCLONAL GAMMAPATHY

A Case Report with Immunofluorescence Study of Bone Marrow and Spleen

Ingemar Turesson and Alf Rausing

*From the Departments of Internal Medicine and Pathology, University of Lund
Malmö General Hospital, Malmö, Sweden*

Abstract: A case of chronic non-neuronopathic Gaucher disease is presented. The diagnosis was based on the finding of typical Gaucher cells in the bone marrow and spleen. The patient also had a benign monoclonal gammopathy. Laserdiffraction studies of bone marrow and spleen were performed using monospecific antiserum against immunoglobulin heavy and light chains. The results indicate a monoclonal plasma cell proliferation in the bone marrow whereas the distribution of plasma cells in the spleen was polyclonal. The combination of chronic Gaucher's disease and monoclonal gammopathy was also found in the patient's brother.

The lipid storage disease first described by Gaucher in 1882 (6) is characterized by accumulation of glucosyl ceramide in reticuloendothelial cells of various tissues, particularly the liver, spleen and bone marrow. Two clinical forms can be distinguished: neuronopathic Gaucher's disease, which can be acute or subacute and is seen in infants and young children, and chronic non-neuronopathic Gaucher's disease affecting older children and adults (4). The chronic form has a much more protracted course and clinical manifestations may be absent until late adult life. It is also much more common (10-20 times) than the infantile type (1). Chronic Gaucher's disease is generally seen in Ashkenazy Jews. It is a hereditary condition and the autosomal recessive nature of the disease is well established (5).

The biochemical basis of the disease is deficient activity of the enzyme glucocerebrosidase (?). This leads to ineffective breakdown of various glycolipids and excessive accumulation of glucosyl ceramide in reticuloendothelial cells. They take on a characteristic appearance with a wrinkled or

striated cytoplasm and have been named Gaucher cells. The most common clinical symptoms are due to accumulation of such cells in the liver and spleen, leading to hepato- and splenomegaly and clinical signs of hypersplenism (4). Bone marrow involvement is common and typical roentgenographic bone lesions are seen in 50-75% of the cases (4). Other common symptoms are pigoeculae and yellow-brown skin pigmentation. A high level of non-tartrate-inhibitable serum acid phosphatase is helpful in making the diagnosis (17), which is confirmed by the presence of Gaucher cells in bone marrow aspirates. An interesting aspect of chronic Gaucher's disease is the occurrence of serum immunoglobulin abnormalities. These include both polyclonal immunoglobulin increase and monoclonal gammopathy (7, 13, 15, 16, 19, 21).

We report a case of Gaucher's disease with a monoclonal gammopathy without clinical signs of multiple myeloma. By immunofluorescence studies the M-component producing cell clone was traced to the bone marrow but not to the spleen.

CASE REPORT

The patient is a 55-year-old Ashkenazy Jewish woman who was referred to the Department of Medicine in 1968 because of fatigue and arthralgia. Her past history included operation for inguinal hernia, appendectomy and cholecystectomy. The physical examination revealed nothing abnormal. There were no pigoeculae, no abnormal pigmentation and no hepato- or splenomegaly. She had moderate normochromic anemia and slight leukopenia and thrombocytopenia. Hb level was 10.1 g/100 ml, RBC 4.9 mil./mm^3 , WBC 3100 mm^3 and platelets $110000/\text{mm}^3$. ESR was 37 mm/h. Plasma protein analysis using agarose gel electrophoresis, immunoelectrophoresis and

monoclonal gammopathy splenic tissue was examined post mortem. The distribution of immunoglobulin-containing cells was polyclonal. The bone marrow was however not investigated.

An alternative explanation is a common genetic predisposition to Gaucher's disease and immunoglobulin disturbances. Chronic Gaucher's disease is known to be inherited as an autosomal recessive trait (5). It would be interesting to study the incidence of polyclonal and monoclonal hypergammaglobulinemia in relatives of patients with this disease. As far as we know no such study has been performed. The coexistence of Gaucher's disease and an M-component of the same immunoglobulin class in our patient's brother is noteworthy. Further characterization of the M-components including use of anti-idiotypic antisera is intended.

ACKNOWLEDGEMENTS

The investigation was supported by grants from Alfred Österlund Foundation and from Cancer Research Funds of Malmö General Hospital, Malmö, Sweden.

REFERENCES

- Blattner R J. Gaucher disease. Abnormalities in immunoglobulins. *J Pediatr* 73: 626, 1968.
- Brady R O, Kanfer J N & Shapiro D. The metabolism of glucocerebrosides. II. Evidence of an enzymatic deficiency in Gaucher's disease. *Biochem. biophys. Res. Commun.* 18: 221, 1965.
- Fisher E R & Rendborg H. Gaucher disease. Pathogenetic considerations based on electron microscopic and histochemical observations. *Amer J Pathol* 41: 679, 1962.
- Fredrickson D S & Sloan H R. Glucosyl ceramide lipidoses. I. The metabolic basis of inherited disease (ed. J B Stanbury, J B Wyngaarden and D S Fredrickson). McGraw-Hill, New York, 1977.
- Fried I. Population study of chronic Gaucher's disease. *Israel J med Sc* 9: 1396, 1973.
- Gaucher P C E. De l'épithéliom de la rate. Hypertrophie kaposiiforme de la rate sans leucémie. Thèse de Paris 1882.
- Goldfarb A R., Atlas D H & Gaherman. Electrophoretic studies in Gaucher's disease. *J clin. Path* 20: 963, 1970.
- Hijmans W., Schuit, H. R. E. & Huhling-Hessink E.. An immunofluorescence study on intracellular immunoglobulins in human bone marrow cells. *Ann. Y Acad Sci.* 177: 290, 1971.
- Hijmans W., Schuit, H. R. E., Jongema, A. P. M., Ploem, J. S. Performance testing of sera against human immunoglobulins. In: *utilization in immunofluorescence* (ed. E. J. borow). Blackwell, Oxford and Edinburgh, 1970.
- Hijmans, W., Schuit, H. R. E. & Klein, F. A. An immunofluorescence procedure for the detection of intracellular immunoglobulins. *Clin. exp Immunol* 437: 1969.
- Lake B D. A histochemical study of Gaucher's disease and Niemann-Pick disease. *J roy micr* 86: 417, 1966.
- Laurell C-B. Electrophoretic and immunochemical analysis of proteins. *Scand J clin. Lab I Suppl* 124, 1972.
- Osserman E. F. & Takatsuki K. Plasma myeloma, gamma globulin synthesis and. *Medicine* 42: 337, 1963.
- Petruselli M., Scarsvilli, F. & Zacheo F. I. morphogenesis of Gaucher cells investigated electron microscopy. *Blood* 34: 331, 1969.
- Pinkhas, J., Djalili M. & Yarow M. Coincidence of multiple myeloma with Gaucher's disease. *med Sci* 1: 537, 1965.
- Pratt, P. W., Euren S. & Hochman, S. Immunoglobulin abnormalities in Gaucher's disease. Report of cases. *Blood* 31: 633, 1968.
- Tuchman, L. R., Goldstein G. & Chyman. Studies on the nature of the increased serum phosphatase in Gaucher disease. *Amer J M* 27: 959, 1959.
- Thresson I. Distribution of immunoglobulin containing cells in human bone marrow and its tissue I. preparation.
- Tyson M. C., Grossman W. I. & Tuchman, L. Gaucher's disease (with elevated serum acid phosphatase level) masquerading as cirrhosis of liver. *Amer J Med* 37: 156, 1964.
- Waldenström J. Benign monoclonal. In: *Multiple myeloma and related disorders* (ed. Azar and M. Potter). Harper and Row, New York, 1973.
- Wolf P. Monoclonal gammopathy in Gaucher disease. *Lab Med* 4: 28, 1973.

